°FORM PTO-1390 OFFICE (REV 11-2000)	U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK	ATTORNEY'S DOCKET NUMBER: 500852000101
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/869002
INTERNATIONAL APPLICATION NO.: **PCT/US99/31230**	INTERNATIONAL FILING DATE: **29 December 1999 (29.12.99)**	PRIORITY DATE CLAIMED: **29 December 1998 (29.12.98)**

TITLE OF INVENTION

HEAT TOLERANT BROCCOLI

APPLICANT(S) FOR DO/EO/US

BARHAM, Robert and JOYNT, David

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau) **(including Request, Description, Claims, Absract, Drawing)**
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application under PCT Article 19 (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) **(signed)**.
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 19. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☒ A copy of the International Search Report (PCT/ISA/210) and cited references.
15. ☒ A copy of the published International Application.
16. ☒ A copy of Form PCT/IPEA/402..
17. ☒ A copy of the Written Opinion (Form PCT/IPEA/408).
18. ☒ A copy of the International Preliminary Examination Report (Form PCT/IPEA/416).
19. ☒ Other items or information: Copies of Forms PCT/IB/301, 304, 306, 308; and return receipt postcard.

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

Express Mail Label No.: **EL 568 250 172 US** Date of Deposit: **June 22, 2001**

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to: Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231, Attention: United States Designated/Elected Office (DO/EO/US)..

Elizabeth A. Reicker

Elizabeth A. Reicker

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) *	INTERNATIONAL	ATTORNEY'S DOCKET
09/869002	APPLICATION NO PCT/US99/31230	NUMBER. 500852000101

21. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provision of PCT Article 33(1)-(4) $690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00

	CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$690.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ .00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	28 - 20 =	8	x $18.00	$690.00	$144.00	
Independent claims	4 - 3 =	1	x $80.00	$ 80.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$ .00	
TOTAL OF ABOVE CALCULATIONS =				$914.00	
☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.				$457.00	
SUBTOTAL =				$457.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ .00	
TOTAL NATIONAL FEE =				$457.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 per property** +				$ 40.00	
TOTAL FEES ENCLOSED =				$497.00	
				Amount to be refunded:	**$***
				charged:	**$***

a. ☒ A check in the amount of $497.00 to cover the above fees is enclosed.

b. ☐ Please charge my **Deposit Account No. 03-1952** in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to **Deposit Account No. 03-1952**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Michael R. Ward, Esq.
Morrison & Foerster LLP
425 Market Street
San Francisco, California 94105-2482

Michael R Ward

SIGNATURE

Michael R. Ward
Registration No. 38,651

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

Express Mail Label No.: **EL 568 250 172 US** Date of Deposit: **June 22, 2001**

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Elizabeth A. Reicker

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Robert BARHAM et al.

Serial No.: TO BE ASSIGNED

Filing Date: HEREWITH

For: HEAT TOLERANT BROCCOLI

Examiner: TO BE ASSIGNED

Group Art Unit: TO BE ASSIGNED

PRELIMINARY AMENDMENT

Box PCT - Attention DO/EO/US
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In advance of prosecution, please enter the following amendments and remarks.

AMENDMENT

IN THE SPECIFICATION

At page 1, line 3, before "Field of the Invention" please insert the following:

--CROSS REFERENCE TO RELATED APPLICATIONS

This application is a U.S. national phase application of International PCT/US99/31230, filed December 29, 1999, which claims priority to U.S. Application Serial No. 09/328,121 filed June 8, 1999, which claims priority to U.S. Provisional Application Serial No. 60/114,038, filed December 29, 1998, all of which are hereby incorporated by reference in their entirety.--

IN THE CLAIMS

Please CANCEL claims 1-53.

Please add the following NEW claims.

--54. (New) A commercially acceptable broccoli plant comprising a center head having a diameter of 3 to 8 inches at maturity when said plant is exposed to a maximum temperature of 90°F for at least 5 consecutive days during the growth cycle of said plant.

55. (New) Seed produced by the plant of claim 54.

56. (New) Progeny seed produced from crossing the broccoli plant of claim 54 with another plant.

57. (New) A commercially acceptable broccoli plant or its parts produced from the seed of claim 54.

58. (New) A commercially acceptable broccoli plant regenerated from tissue culture of the broccoli plant of claim 57.

59. (New) Tissue culture according to claim 58 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

60. (New) A commercially acceptable regenerated broccoli plant regenerated from the regenerable cells of a tissue culture according to claim 59 wherein said regenerated plant comprises a center head having a diameter of 3 to 8 inches at maturity when said plant is exposed to a maximum temperature of 90°F for at least 5 consecutive days during the growth cycle of said plant.

61. (New) A commercially acceptable broccoli plant comprising a center head having a diameter of 3 to 8 inches at maturity when said plant is exposed to a maximum temperature of 95°F for at least 1 day during the growth cycle of said plant.

62. (New) Seed produced by the plant of claim 61.

63. (New) Progeny seed produced from crossing the broccoli plant of claim 61 with another plant.

64. (New) A commercially acceptable broccoli plant or its parts produced from the seed of claim 61.

65. (New) A commercially acceptable broccoli plant regenerated from tissue culture of the broccoli plant of claim 64.

66. (New) Tissue culture according to claim 65 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

67. (New) A commercially acceptable regenerated broccoli plant regenerated from the regenerable cells of a tissue culture according to claim 66 wherein said regenerated plant

comprises a center head having a diameter of 3 to 8 inches at maturity when said plant is exposed to a maximum temperature of 95°F for at least 1 day during the growth cycle of said plant.

68. (New) A commercially acceptable broccoli plant comprising a center head having a diameter of 3 to 8 inches at maturity when said plant is exposed to a maximum temperature of 85°F for at least 15 days during the growth cycle of said plant.

69. (New) Seed produced by the plant of claim 68.

70. (New) Progeny seed produced from crossing the broccoli plant of claim 68 with another plant.

71. (New) A commercially acceptable broccoli plant or its parts produced from the seed of claim 70.

72. (New) A commercially acceptable broccoli plant regenerated from tissue culture of the broccoli plant of claim 71.

73. (New) Tissue culture according to claim 72 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

74. (New) A commercially acceptable regenerated broccoli plant regenerated from the regenerable cells of a tissue culture according to claim 73 wherein said regenerated plant comprises a center head having a diameter of 3 to 8 inches at maturity when said plant is exposed to a maximum temperature of 85°F for at least 15 days during the growth cycle of said plant.

75. (New) A commercially acceptable broccoli plant comprising a center head having a diameter of 3 to 8 inches at maturity when said plant is exposed to a maximum temperature of 80°F for at least 20 days during the growth cycle of said plant.

76. (New) Seed produced by the plant of claim 75.

77. (New) Progeny seed produced from crossing the broccoli plant of claim 75 with another plant.

78. (New) A commercially acceptable broccoli plant or its parts produced from the seed of claim 75.

79. (New) A commercially acceptable broccoli plant regenerated from tissue culture of the broccoli plant of claim 78.

80. (New) Tissue culture according to claim 79 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

81. (New) A commercially acceptable regenerated broccoli plant regenerated from the regenerable cells of a tissue culture according to claim 80 wherein said regenerated plant comprises a center head having a diameter of 3 to 8 inches at maturity when said plant is exposed to a maximum temperature of 85°F for at least 15 days during the growth cycle of said plant.

REMARKS

Prior to Examination on the merits, applicants request entry of the above amendments. Claims 1-53 are canceled. New claims 54-81 are added. The claims are supported in the application as filed. In particular, the Examiner's attention is directed to page 2 lines 20-30, page 5 lines 19-30 and page 6, lines 1-2.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

Early and favorable examination is requested.

Respectfully submitted,

Dated: June 22, 2001

By: [signature]

Michael R. Ward
Registration No. (38,651)

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"**Version with markings to show changes made**"

HEAT TOLERANT BROCCOLI

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a U.S. national phase application of International PCT/US99/31230, filed December 29, 1999, which claims priority to U.S. Application Serial No. 09/328,121 filed June 8, 1999, which claims priority to U.S. Provisional Application Serial No. 60/114,038, filed December 29, 1998, all of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

This invention is in the field of plant breeding. In particular, this invention relates to the development of heat tolerant broccoli (*Brassica oleracea* L. var. *italica*).

BACKGROUND OF THE INVENTION

Broccoli (*Brassica oleracea* L. var. *italica*) has become an increasingly popular crop worldwide especially in health-conscious areas of the western world such as the North America, Europe, and Japan. An average broccoli stalk contains only 30 calories and provides 240% of the recommended daily allowance of vitamin C plus 10% of the recommended daily allowance of vitamin A. In addition to its nutritional value, some recent studies have shown that broccoli aids in the prevention of some forms of cancer.

Broccoli is a cool weather crop. High temperatures (>80°F) for even relatively short periods of time and warm temperatures (>75°F) for extended periods of time cause broccoli heads to be rough with uneven flower bud sizes and thus commercially unacceptable. {(Björkman, T., et al. (1998) High temperature arrest of inflorescence development in broccoli (*Brassica oleracea* var. *italica* L.) Journal of Experimental Botany 49:101-106.} As a result of the high sensitivity to heat during growth, broccoli can only be grown in limited areas under cool weather conditions.

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Previous attempts at identifying heat tolerant broccoli cultivars have not been successful because broccoli is sensitive to relatively short periods of heat stress thereby making field observations too variable for effective genetic screening. Björkman, et al. (1998).

Thus, there is a need to develop heat tolerant broccoli varieties that will produce commercially acceptable broccoli heads under warm weather heat stress growth conditions. In addition, there is a need to develop heat tolerant broccoli inbred lines useful for producing heat tolerant F1 seed.

SUMMARY OF THE INVENTION

In order to meet these needs, the present invention is directed to heat tolerant broccoli plants. In particular, this invention is directed to broccoli seed capable of germinating and growing into a plant capable of producing a commercially acceptable head under heat stress growth conditions.

The broccoli seed of this invention are capable of germinating into a plant capable of producing a commercially acceptable head under heat stress growth conditions that render the heads of commercially available broccoli commercially unacceptable.

In addition to being heat tolerant, the broccoli seed of this invention are capable of germinating into a plant that is predominately mildew resistant.

The broccoli seed of this invention will produce a plant with a commercially acceptable head when the plant is exposed to a maximum temperature of 90°F for at least 5 consecutive days during the growth cycle; when the plants are exposed to a maximum temperature of at least 95°F for at least one day during the growth cycle; when the plants are exposed to a maximum temperature of 85°F for at least 15 days during the growth cycle; when the plants are exposed to a maximum temperature of at least 75° for at least 25 days during the growth cycle; when the plants are exposed to a maximum temperature of at least 80°C for at least 20 days during the growth cycle and other heat stress growth conditions.

The broccoli seed of this invention include but are not limited to those seeds designated M7028, M7007, M7009, M7022, 393-2-19, H7008, H7022, 393-2-47, 98-2192, 98-2088, 98-2061, H7007, H7028, H7010, and H7021R. The broccoli seed of this invention further include lines 4243, 4221, 4441, 4274-1, 4274-2, 4278-1, 4284-1, 4285-1, 4354-1, 4354-2, 4377-1, 4318-1, 4320-1, 4320-2, 4321-1, 4437-1, 4476-1, 4462-1, 4308-2, 4309-1, 4355-1, 4412-1, 4301, 4303, 4304, 4317, 4468, 4470, 4471, 4263-1, 4430-1, 4450-1, 4450-2, 4432-1, 4267-1, 7861, 7864, 7865, 7881, 7887, 7935, 8092, 7883, 7914, 7770, 7778.

The broccoli seed of this invention further include lines 4201, 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1, 4415, 4418, 4395-2.

Each of the lines of this invention can be crossed with other broccoli lines.

The broccoli seeds of this invention include inbred lines, hybrid lines, male lines and female lines, all of which are heat tolerant and capable of producing a commercially acceptable head under heat stress growth conditions.

This invention is further directed to broccoli plants or parts of broccoli plants produced from the broccoli seed of the invention. The invention is further directed to broccoli plants regenerated from tissue culture of the broccoli plants of this invention. The tissue culture of the invention comprises regenerable cells including meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen and plants regenerated from these cells.

The invention is further directed toward transgenic heat tolerant broccoli plants. The transgenic heat tolerant broccoli lines may be resistant to various herbicides or pesticides.

The invention is further directed to broccoli plants having all of the phenotypic characteristics of the plants produced from the heat

tolerant broccoli seed of the invention. The invention is further directed to plants resulting from selecting, crossing, breeding or otherwise altering the broccoli plants of this invention.

The invention is further directed to biological material isolated from the plants of this invention. Such material includes but is not limited to RNA, DNA, protein and carbohydrate. The DNA of these plants includes the gene(s) involved in heat tolerance.

This invention is further directed to the seeds and plants produced from crossing other broccoli lines with plants grown from the seed of this invention.

This invention is further directed to methods of breeding heat tolerant broccoli lines.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be better understood by reference to the drawings in which:

Figure 1 shows various broccoli head shapes. Broccoli heads are referred to as domed, semi-domed and deep-domed. The shapes of the various domes are (1) circular; (2) transverse broad elliptic; (3) transverse elliptic and (4) transverse elliptic narrow.

Figure 2 shows a cross section of eight 40-inch beds utilized for field production of the hybrid seed of this invention. In this diagram: F = female line seed-line; M1 = first male planting seed-line; M2 = second male planting seed-line; M3 = third male planting seed-line and M4 = fourth male planting seed-line.

DETAILED DESCRIPTION OF THE INVENTION

In order to more completely understand the invention, the following definitions are provided.

Broccoli: Broccoli (*Brassica oleracea* L. var. *italica*) is a cool season vegetable in the mustard family. Principal broccoli varieties currently grown in California include, in the coastal valleys, Everest, Greenbelt, Legacy, Marathon, Ninja, Olympia, Pinnacle, Pirate, Republic, Shogun, and Sultan; in the desert valleys, Arcadia, Captain, Emperor, Everest, Galaxy, Galleon, Greenbelt, Major, Marathon, Ninja, Packman, Patriot, Pirate, and Sultan; and in the San Joaquin Valley, Arcadia, Captain, Everest, Greenbelt, Legacy, Legend, Marathon, Pirate, and Republic. Varieties grown in the Pacific Northwest are: Arcadia, Emerald City, Excelsior, Pakman Patriot, Pirate, Regal, Arcadia, Buccaneer, Emerald City, Emperor, Everest, Excelsior, Green Belt, Green Valiant, Laguna, Legend, Liberty, Major, Marathon, Pakman, Patriot, Pinnacle, Pirate, Premium Crop, Regal, Shogun, Samurai, Triathlon, Windsor, Barbados, Embassy, Green Comet, Green Defender, HMX 1134, Idol. Because of heat sensitivity, broccoli is typically grown for harvest in the spring and fall.

Commercially Acceptable Broccoli: Commercially acceptable broccoli is broccoli which vegetable growers/shippers find acceptable for sale and consumers find acceptable for personal purchase and, ultimately, human consumption. Commercially acceptable broccoli has small uniform beads, good blue-green to green color, and tight, dome-shaped heads that extend above the leaves for ease of harvest. In commercial plantings under optimum conditions, large leafy broccoli plants produce a compact flower head on a tall, green, branching stalk. The center flower head is from 3 to 8 inches (7.5-20 cm) in diameter and plants average 24 to 36 inches (60-90 cm) tall. Hollow stems, water head rot, brown or yellow beads, bracts (leaflets) within heads, uneven bead size, and excessive branching

are undesirable and commercially unacceptable defects in broccoli that can be caused by exposure to heat.

Heat Tolerant Broccoli: Heat tolerant broccoli is broccoli that will produce a commercially acceptable product when grown under heat stress growth conditions for broccoli.

Heat Stress Growth Conditions: Heat stress growth conditions for broccoli are elevated temperature growth conditions that result in broccoli that exhibit heat stress symptoms that result in a commercially unacceptable product. Heat stress symptoms include non-uniform beads; brown, yellow, light-green or purple colored heads; loose flat heads; prominent leaflets that come through the broccoli head as bracts; hollow stems; water head rot and excessive branching.

Single Plant Selection: Single plant selection is the process of selecting single plants, which exhibit desired traits or characteristics. Seeds from the single plant are collected, stored and then grown in a subsequent growing period for further selections.

Massed: Broccoli plants are massed when a number of plants are selected and brought together for cross-pollination as a group. Massing prevents further inbreeding and tends to "fix" the broccoli line at the stage from which the selections were made.

Self-Pollination/Self-Pollinator: Self-pollination is the process of putting pollen from a plant onto a receptive female flower-part of that same plant. A plant that is a self-pollinator is a plant that accepts its own pollen to make seed that typically will give rise to

plants very similar or the same as the self pollinator plant. A plant that is self-pollinated is said to be selfed.

Self-Incompatible: A self-incompatible plant will not, under normal conditions, accept its own pollen nor generate any self-seed. Self-incompatible lines are generally designated "female." Self-incompatible lines are generally crossed with other lines to produce hybrid seed.

Self-Compatible: A self-compatible plant accepts its own pollen and will produce self-seed. Self-compatible lines are generally designated "male."

Progeny: Progeny is a broccoli line that is the offspring of the previous generation broccoli line.

Sessile: Attached to the stem by the base of the leaf.

Petiolate: Attached to the stem via a petiole.

Hybrid: The progeny of cross-fertilization between parents belonging to different genotypes.

Hybrid Vigor: The phenomenon in which the cross of two parent stocks produces hybrids that show increased vigor/heterosis compared to the parent stocks.

Inbred Lines: A nearly homozygous line produced by continuous inbreeding.

Pedigree Breeding: A system of breeding in which individual plants are selected in the segregating generations from a cross on the basis of their desirability and on the basis of a pedigree record.

The terminology used to describe the broccoli plants of this invention are generally those used by the Plant Variety Protection Office in PVP form STD-470-44 "Objective Description of Variety Broccoli (*Brassica oleracea* var. *italica*)." The following terminology is used herein in comparative study #1 and comparative study #2.

1. REGION OF ADAPTATION (Area where best adapted in USA):

(1) Northwest; (2) NorthCentral; (3) Northeast; (4) Southeast; (5) Southwest; (6) Most regions and (7) Pacific Coast.

2. MATURITY (Main Crop at 50% Harvest):

Harvest Season: (1) Fall; (2) Fall/Winter; (3) Winter/Spring; (4) Spring/Summer; (5) Summer; and (6) Summer/Fall.

Spring Planted: (1) Days from Direct Seeding to 50% Harvest; (2) Days from Transplanting to 50% Harvest; and (3) Length of Harvest Period in days.

Fall Planted: (1) Days from Direct Seeding to 50% Harvest; (2) Days from Transplanting to 50% Harvest; and (3) Length of Harvest Period in days.

Time of beginning of flowering (50% of plants with at least 10% flowers: (1) Early; (2) Med-Early; (3) Medium; (4) Med-Late; and (5) Late.

3. SEEDLING:

Cotyledon Color: (1) Yellow-Green; (2) Light Green; (3) Medium Green; (4) Dark Green; (5) Blue-Green; and (6) Purple-Green.

Cotyledon Anthocyanin: (1) Absent; (2) Weak; (3) Intermediate; and (4) Strong.

Hypocotyl Anthocyanin: (1) Absent; (2) Weak; (3) Intermediate; and (4) Strong.

4. **PLANT (At Harvest)**:

Plant Height: (cm) from soil line to top of leaves

Head Height: (cm) from soil line to top of head

Plant Branches: (1) Few; (2) Medium; and (3) Many.

Plant Habit: (1) Spreading; (2) Intermediate; and (3) Compact.

Market Class: (1) Fresh Market; (2) Processing; and (3) Both

Life Cycle: (1) Annual; (2) Biennial; and (3) Perennial.

Type of Variety: (1) Inbred; (2) Open-Pollinated; and (3) First generation Hybrid.

5. **OUTER LEAVES (at Harvest)**:

Number of Leaves per Plant:

Width at midpoint of plant including petiole:

Length at midpoint of plant including petiole:

Petiole Length:

Leaf Ratio-Length/Width: (1) (2:1); (2) (3:1); (3) (4:1); (4) (5:1); and (5) (6:1).

Leaf Attachment: (1) Sessile; (2) Petiolate; and (3) Sessile and Petiolate (both).

Wax Presence: (1) None; (2) Weak; (3) Intermediate; and (4) Strong.

Foliage Color (with wax if present): 1 Light Green; (2) Medium Green; (3) Dark Green; (4) Grey-Green; (5) Blue-Green; and (6) Purple-Green.

Leaf Shape: (1) Narrow Elliptic; (2) Elliptic; and (3) Broad Elliptic.

Leaf Base: (1) Blunt and (2) Pointed.

Leaf Apex: (1) Blunt and (2) Pointed.

Leaf Margins: (1) Straight; (2) Slightly Wavy; and (3) Very Wavy.

Leaf Veins: (1) Thin; (2) Intermediate; and (3) Thick.

Midrib: (1) Not Raised; (2) Slightly Raised; and (3) Raised.

Blistering (1) None; (2) Weak; and (3) Intermediate; and (4) Strong.

Attitude (Leaf Angle from Ground): (1) Horizontal (0-15 degrees); (3) Semi-erect (35-55 degrees); and (5) Erect (80-100 degrees).

Torsion of Leaf Tip: (1) None; (2) Weak; (3) Intermediate; and (4) Strong.

Profile of Upper Side of Leaf: (1) Concave; (2) Planar; and (3) Convex.

6. HEAD (At Market Maturity):

Diameter at widest point:

Depth:

Weight: market trimmed

Color: (1) Light Green; (2) Medium Green; (3) Dark Green; (4) Blue/Green; and (5) Purple.

Head Shape: (1) Circular; (2) Transverse Broad Elliptic; (3) Transverse Elliptic; and (4) Transverse Elliptic Narrow.

Dome Shape: (1) Domed; (2) Semi-domed; and (3) Deep Domed.

Head Size: (1) Small; (2) Medium; and (3) Large.

Compactness: (1) Long Pedicels (Loose); (2) Medium; and (3) Short Pedicels (Tight).

Surface Knobbling: (1) Fine; (2) Medium; and (3) Coarse.

Beads size: (1) Small; (2) Medium; and (3) Large.

Flower Buds: (1) Even in size; and (2) Uneven in size (cateye).

Anthocyanin Coloration: (1) Absent; 2 Present; (3) Leaf Axils; (4) Leaf Veins; (5) Leaf Blade; (6) Entire Plant; and (7) Leaf Petiole.

Color of Head Leaves: (1) White; (2) Green; (3) Red; and (4) Purple.

Secondary Heads: (1) Completely absent; (2) Basal; (3) Combination; and (4) Axillary along entire main stem up to main head.

Prominence of Secondary Heads: (1) Weak, (2) Intermediate; and (3) = Strong.

Number of Secondary Heads:

7. COLOR:

Flower Color: (1) White; (2) Cream; and (3) Yellow.

Flower Stalk Color: (1) Green; (2) Purple; and (3) Variegated.

8. DISEASE RESISTANCES:

1 = Most Susceptible
5 = Intermediate
9 = Most Resistant

Black Leg (*Leptosphaeria maculans*)	Black Leg
Black Spot (*Alternaria* spp.)	Black Spot
Black Rot	Black Rot
Bottom Rot (*Rhizoctonia solani*)	Bottom Rot
Cauliflower Mosaic Virus	Cauliflower Mosaic Virus
Cerospora Leaf Spot (*Cercospora brassicicola*)	Cerospora Leaf Spot
Clubroot (*Plasmodiophora brassicae*)	Clubroot
Downy Mildew (*Peronospora parasitica*)	Downy Mildew
Erwinia Sp.	Erwinia Sp.
Phytophthora Root Rot (*Phytophthora megasperma*)	Phytophthora Root Rot
Powdery Mildew (*Erysiphe polygoni*)	Powdery Mildew
Pseudomonas	Pseudomonas
Ring Spot (*Mycosphaerella brassicicola*)	Ring Spot

Turnip Yellow Mosaic Virus	Turnip Yellow Mosaic Virus
Verticillium wilt (*Verticillium albo-atrum*)	Verticillium wilt
White Blister (*Albugo candida*)	White Blister
Xanthomonas campetis	Xanthomonas campetis
Yellows (*Fusarium oxysporum*)	Yellows

9. OTHER RESISTANCE:

1 = Most susceptible
5 = Intermediate
5 9 = Most Resistant

Insect
Buttoning
Blindness
Bolting
Brown beads
Drought
Cold
Hollow Stem
Riceyness
Whiptail

10. HEAT TOLERANCE:

Heat tolerance was measured on a scale of 1-9 with 9 being
10 the most heat tolerant and 1 being the least heat tolerant. For heat tolerance, ratings of five (5) or below are indicative of a broccoli plant that produced a commercially unacceptable head. A rating of six (6) is indicative of broccoli plants that exhibit no heat stress symptoms when exposed to 90°F. A rating of seven (7) is
15 indicative of broccoli plants that exhibit no heat stress symptoms when exposed to 95°F. A rating of eight (8) is indicative of broccoli plants that exhibit no heat stress symptoms when exposed to 100°F. A rating of nine (9) is indicative of broccoli plants that exhibit no heat stress symptoms when exposed to 105°F. In some
20 studies, plants were not exposed to a temperature of 105°F so that a rating of 9.0 could not be assigned.

Under some circumstances, the heat tolerant ratings are followed by a (+) or (-) to indicate a plant exhibiting symptoms

slightly better (+) or slightly worse (-) than the assigned number. In other circumstances, the ratings are presented as a fraction of a rating number. For example, a rating of 7.1 is slightly better than a rating of 7.0. A rating of 7.5 is half way between a rating of 7.0 and 8.0. A rating of 6.8 is slightly worse than a rating of 7.0. A slightly higher rating means that the heat stress symptoms were slightly less evident. Similarly, a slightly lower rating means that the heat stress symptoms were less evident.

Taking into account these definitions, the present invention is directed to heat tolerant broccoli plants. The heat tolerant broccoli of this invention is capable of producing a commercially acceptable product when grown under heat stress conditions.

Heat stress is exhibited in broccoli by a number of different symptoms. These symptoms include non-uniform beads; brown, yellow, light-green or purple colored heads; flat heads; bracts (leaflets in the head); rapid fracturing of the head, which reduces the harvest period; "cateye" (death of growing points), extremely small heads, and hollow stems.

Each of these symptoms is generally viewed as commercially unacceptable. The greater the number of heat stress symptoms, the more commercially unacceptable the broccoli plant. Heat stress symptoms in broccoli result from a number of interacting factors. The most important of these interacting factors are the temperature, the duration of the high temperature exposure (hours, days, weeks), the available soil moisture supply and the wind speed. Of critical importance is the timing of the exposure to the heat stress conditions during the growth cycle of broccoli. It has been shown that heat stress of broccoli can be due to an inhibition of the enlargement of broccoli bud primordia. Broccoli buds are not as sensitive to heat once they differentiate. The different heat sensitivity and resulting contrast between the delayed buds and the

unaffected buds causes the uneven head appearance under heat stress growth conditions. If heat stress occurs prior to bud development (i.e., during vegetative development) no injury is generally seen. If the heat stress is applied late in bud development, many buds are affected but these buds can be obscured by the older buds.

Because of extreme sensitivity to heat stress, broccoli grown in the spring and the fall, when cooler temperatures are the norm, are at less risk of heat stress. However, a single day during the spring or fall with a high temperature of 100°F or several (2-3) warmer days (>80°F) or multiple (5-7) warm days (>75°F) at the critical point during broccoli bud development can render an entire field so damaged by heat stress that none of the heads are commercially acceptable.

The present invention is thus directed toward the development of heat tolerant broccoli varieties and hybrids. The broccoli varieties and hybrids of this invention will produce commercially acceptable heads when the plants are grown during heat stress growth conditions during late spring, summer, and early fall in California, Arizona, Mexico, and many other areas traditionally considered to be too warm for broccoli growth, or at risk of heat stress.

Broccoli Production

Broccoli may be grown by transplant production or by direct seeding. For transplant production, plants may be started in hotbeds or greenhouses. Broccoli seedlings grown in a hotbed need a loose, easily pulverized loam that is not too fertile. If the plants are started in hotbeds, soil fumigation is needed to control weeds, soil borne diseases, and insects. Seeds are planted one-quarter to one-half inch deep in rows 4 to 6 inches apart with 2 to 4 seeds per inch and covered with a sash or plastic covering. The seedlings are thinned

at the two-leaf stage allowing 1½ inches between plants. Plants are watered twice daily and fertilized with soluble fertilizer at least every 2 weeks. Proper ventilation is important and can be maintained by raising the sash or plastic covering during the hottest portion of the day. In the hotbed, if properly handled, 3 or 4 ounces of seed will produce enough seedlings to plant 1 acre. When seed is planted in beds, it generally requires about 6 to 8 weeks from seed to plants for the spring crop, and about 4 to 5 weeks for the fall crop.

In the greenhouse, a variety of plant growing containers may be used (i.e. plastic cell packs, peat pots, and speedling trays) for growing broccoli. These containers can be filled with an artificial media, usually a combination of peat, perlite, vermiculite, and in some instances bark. The seeds can be sown directly into the containers and thinned upon emergence to 1 plant per cell or pot. In the greenhouse, it generally requires 5 to 6 weeks from seed to plants for the spring crop and 4 to 5 weeks for the fall crop.

For direct seeding, broccoli seeds may be direct seeded in the field using a precision planter. Seed required for one acre is generally 0.75 to 1.25 pound when using a precision seeder.

ORIGIN AND PARENTAGE OF HEAT TOLERANT BROCCOLI PLANTS

The broccoli of this invention were created by classical plant breeding as well as anther culture techniques. The breeding history of the "inbred," "self compatible" and "male" lines identified are exemplified by the following breeding histories.

A. DEVELOPMENT OF INBRED LINES

Numerous heat tolerant inbred lines were developed. For illustrative, but nonlimiting purposes, the breeding histories of the following inbred lines are presented. Unless otherwise noted, single

plant selections were made each year for plants exhibiting heat tolerance.

1. **Inbred Lines 393-2-19, 393-2-47, and 393-2-32**

All lines designated "393-2-XX" where XX represents a different number for a line were isolated and developed as indicated below. Representative lines include 393-2-19, 393-2-47, 393-2-32.

Fall, Year 1	Commercial broccoli hybrid Marathon was crossed with broccoli hybrid "No. 608" obtained from IM Foods, Incorporated, Gilroy, California.
Summer, Year 2	F1 seed from Marathon x No. 608 were planted into row number 393 of a summer broccoli trial in Gilroy, California, and single plant selections were made for heat tolerance and downy mildew resistance.
Fall, Year 2	Single plant selection number 2 from row 393, i.e. "393-2", which had exhibited good heat tolerance and downy mildew resistance, was entered into anther culture.
Spring, Year 3	Anther culture product numbers 19 and 47 from 393-2, i.e."393-2-19," "393-2-47" and "393-2-32" were transplanted into the greenhouse in Gilroy, California. 393-2-19, 393-2-47 and 393-2-32 were observed to exhibit desirable horticultural traits for deep dome-shaped head, lack of side shoots, good vigor, and high yield. 393-2-19, 393-2-47 and

393-2-32 also demonstrated ability to produce self-pollinated seed.

Summer, Year 4	The original seed from 393-2-19, 393-2-47 and 393-2-32 made in the greenhouse in Spring, year 3, were seeded in the greenhouse in Summer, year 4, and subsequently transplanted to the field for evaluation in Gilroy, California, in the summer. 393-2-19 and 393-2-47 exhibited outstanding uniformity and were considered breeding true as a spontaneously doubled-haploid, "inbred", line. Plants were taken from the field plot for self-pollination and subsequent seed increase.
From Year 5 to Present	393-2-19, 393-2-47 and 393-2-32 have consistently exhibited exceptionally good uniformity and stability through generations of seed increase with no variants or off types plants ever observed.

In the breeding history described above, commercial broccoli hybrid Marathon was crossed with broccoli hybrid No. 608 obtained from IM Foods, Incorporated, Gilroy, California. The commercially available broccoli hybrid Marathon was selected for the initial cross because it had demonstrated good yield potential. Hybrid No. 608 was selected for the initial cross because it was thought to have less side shoots, an advantageous characteristic for harvesting.

During the breeding process, F1 seed from Marathon x No. 608 were planted and grown. Selections were made for heat tolerance and downy mildew resistance. The heat tolerance

selection was conducted at head formation through harvest maturity. The selection criteria were smooth domed head, even flower-bud size, good head color, lack of bracting (leaflets in the head), and an ability to hold a good head shape through harvest maturity. The downy mildew selection was conducted throughout the growth cycle of the plants. The selection for downy mildew resistance was based on plants with no mildew lesions or a greatly reduced number of lesions present on any leaves as compared to non-resistant plants.

Multiple single plant selections exhibiting heat tolerance and downy mildew resistance were entered into anther culture. Anther culture procedures are well known in the art of plant breeding. In anther culture techniques, the undifferentiated pollen mother cells that exist in immature broccoli anthers are stimulated *in vitro* into embryonic states by procedures well known in the literature. The undifferentiated pollen mother cells may be subjected to treatments of higher temperatures, light and dark and specialized media growth conditions including hormone simulation. Plant growth conditions of 60°F and bright light followed by a heat shock of 90°F after anther excision and culturing can help stimulate embryogenisis. This process can stimulate the development of embryonic growth wherein the haploid (one-half the chromosome number) pollen mother cell multiplies and grows into callus tissue. The callus tissue, through the use of specialized media, hormone treatments, and controlled temperature and light can be stimulated to make green plant shoots and eventually functional roots. Some of these haploids spontaneously double their chromosome number, thus; generating "di-haploids," which are essentially completely homozygous. These highly homozygous lines are genetically equivalent to the end result of many years of self-pollination by conventional means.

In a preferred format, anther cultures are prepared as described in Keller, et al., Embryogenesis and Plant Regeneration in

Brassica napus Anther Cultures, *Canadian Journal of Botany* 55: 1383-1388 (1977); Keller, et al. Production of Haploids via Anther Culture in Brassica Oleracea Var. Italica, *Euphytica* 32: 151-159 (1983); and Orton, et al., Segregation of Genetic Markers among Plants Regenerated From Cultured Anthers of Broccoli (Brassica oleracea var. 'italica') *Theor Appl Genet* 69:637-643 (1985).

In one format, broccoli anthers are prepared and cultured as follows. Immature broccoli racemes are removed from broccoli heads as they begin to elongate, but before the first buds are opened. Racemes are then sterilized, for example in 20% W:V household bleach for 15 minutes under agitation with one drop of detergent per 100ml as a surfactant. The racemes are then washed at least three times with sterile distilled water for generally 10 minutes per wash. Anthers are then generally removed by carefully peeling back the immature calyx and corolla and gently rupturing the point of filament attached to the anther axis. Care must be taken to minimize damage to the anther culture. Anthers are then placed into liquid culture medium as described in Keller, et al. (1977). Such culture medium may include L-serine at a concentration of 100mg/l. Anthers are cultured at, for example, 35°C for 36h and transferred to 25° (all in the dark). In some circumstances, anther-derived embryos are then kept in continuous fluorescent light (25°C) for 1 week to permit greening and then transferred to hormone-free solidified B_5 medium as described by Gamborg, et al. *Exp. Cell Res.* 50: 151-158 (1968). Upon transfer to the hormone-free medium, the anther-derived embryos are cultured at 25°C in light. Embryos which fail to develop into plantlets may be cut into sections and cultured on a modified Murashige and Skoog medium [Murashige, et al., *Physiol. Plant* 15: 473-497 (1962)] containing 0.8% agar, 2% sucrose, 5 X 10^{-6} M benzyladenine and 10^{-7} M napthaleneacetic acid (NAA) to induce shoot regeneration. To induce rooting, developing

shoots are excised and cultured on B_5 medium in, for example, 60ml sterilin bottles. Rooted plantlets may then be planted in Jiffy-7 peat pellets and kept moist in a mist chamber. After 2 weeks, the broccoli plants can then be transferred to soil and grown in the greenhouse for further selections.

Once transferred to the greenhouse, various plants were selected based on desired phenotypical characteristics including an ability to produce self-pollinated seed. Self-pollination is advantageous because it permits seed increase and bulking of seed without random cross-pollination.

2. **Inbred lines: 4243, 4221 and 4441**

Using the procedures generally outlined above for 393-2-19 and 393-2-47, inbred lines 4243, 4221 and 4441 were isolated following the pedigree chart outlined below. IM Foods 608, Cruiser, Marathon and Sprinter are commercially available lines. Unless otherwise noted, all selections were single plant selections for heat tolerance. 393-2-19 is the same inbred line described above. Each season, the single plant selection exhibiting heat tolerance were selfed and seed was harvested for the next growing season.

Derivation of: 4243; 4221; and 4441

Year 1	IM Foods 608 X Cruiser	and	Marathon X IMF 608
	↓		↓
Year 2	Backcross F_1 to Cruiser		Self
	↓		↓
Year 3	Backcross BC_1 F_1 and Cruiser		F_2 Selection goes to Anther Culture
	↓		↓

	Year 4	Line 5334-1 X Sprinter F_1		393-2-19 (a di-haploid anther culture product)
		↓		
5	Year 5	Line 5526-1	X	393-2-19
			↓	
	Year 6		Self	
			↓	
	Year 7		Self	
10			↓	
	Year 8		Self	
			↓	
	Year 9		Self	
			↓	
15	Year 10		Self	

3. **Inbred lines: 4274-1; 4274-2; 4278-1; 4284-1; 4285-1; 4354-1; 4354-2; 4377-1**

Inbred lines 4274-1; 4274-2; 4278-1; 4284-1; 4285-1; 4354-1; 4354-2; 4377-1 were isolated following the pedigree chart outlined below using the procedures generally described above for inbred lines 393-2-19 and 393-2-47. Unless otherwise noted, single plant selections were made for heat tolerance.

Derivation of: 4274-1; 4274-2; 4278-1; 4284-1; 4285-1; 4354-1; 4354-2; 4377-1

Year 1	IMF608	X	Marathon
			↓
Year 2			Self
			↓
Year 3			Anther Culture
			↓
Year 4			Anther Culture Selection 393-2-47
			↓
Year 5			Unknown Outcross
			↓
Year 6			Self
			↓
Year 7			Self
			↓
Year 8			Self
			↓
Year 9			Self
			↓
Year 10			Self

4. **Inbred lines: 4318-1; 4320-1 and 4320-2; 4321-1; 4437-1; 4476-1; 4462-1**

Inbred lines 4318-1; 4320-1 and 4320-2; 4321-1; 4437-1; 4476-1; 4462-1 were isolated following the pedigree chart outlined below using the procedures generally outlined above for inbred lines 393-2-19 and 393-2-47. Commercially available line Marathon was selfed. Repeated single plant selections for heat tolerance were made to produce the resulting lines. In year 6, the selected line was mass pollinated.

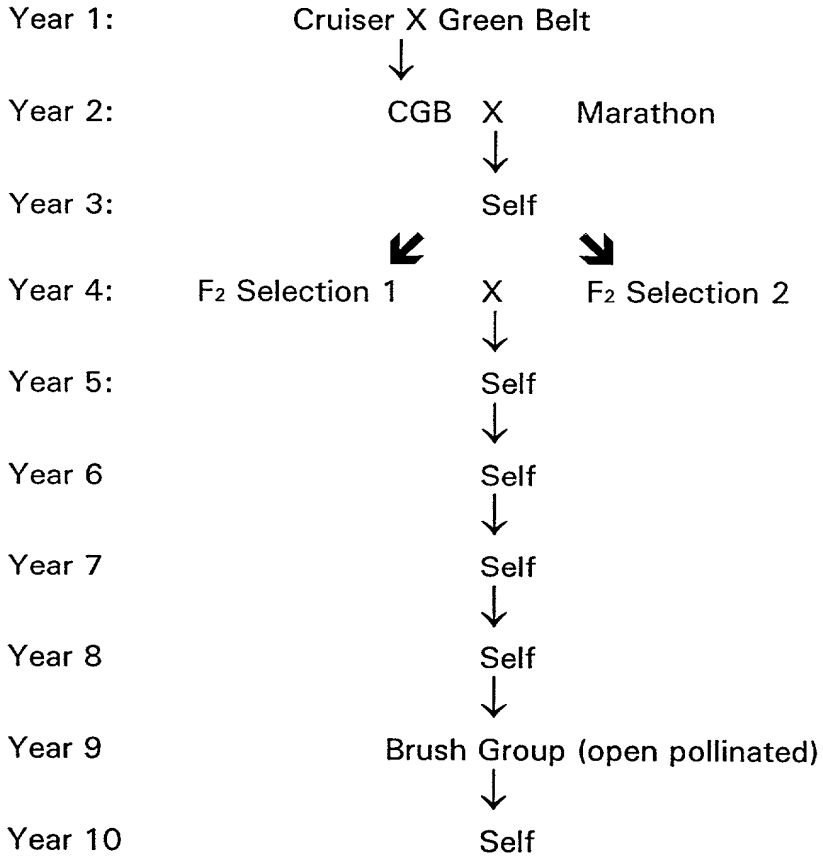
Derivation of: 4318-1; 4320-1 and 4320-2; 4321-1; 4437-1; 4476-1; 4462-1

Year 1	Marathon	Self
		↓
Year 2		Self
		↓
Year 3		Self
		↓
Year 4		Self
		↓
Year 5		Self
		↓
Year 6		Mass Pollinate
		↓
Year 7		Self

5. **Inbred lines: 4308-2; 4309-1; 4355-1; 4412-1; 4301; 4303; 4304; 4317; 4468; 4470; 4471**

Inbred lines 4308-2; 4309-1; 4355-1; 4412-1; 4301; 4303; 4304; 4317; 4468; 4470; 4471 were isolated following the pedigree chart outlined below using the techniques generally outlined above for inbred lines 393-2-19 and 393-2-47. In year 9, the selected lines were brush pollinated, i.e., pollinated with a brush. Single plant selections were made for heat tolerance.

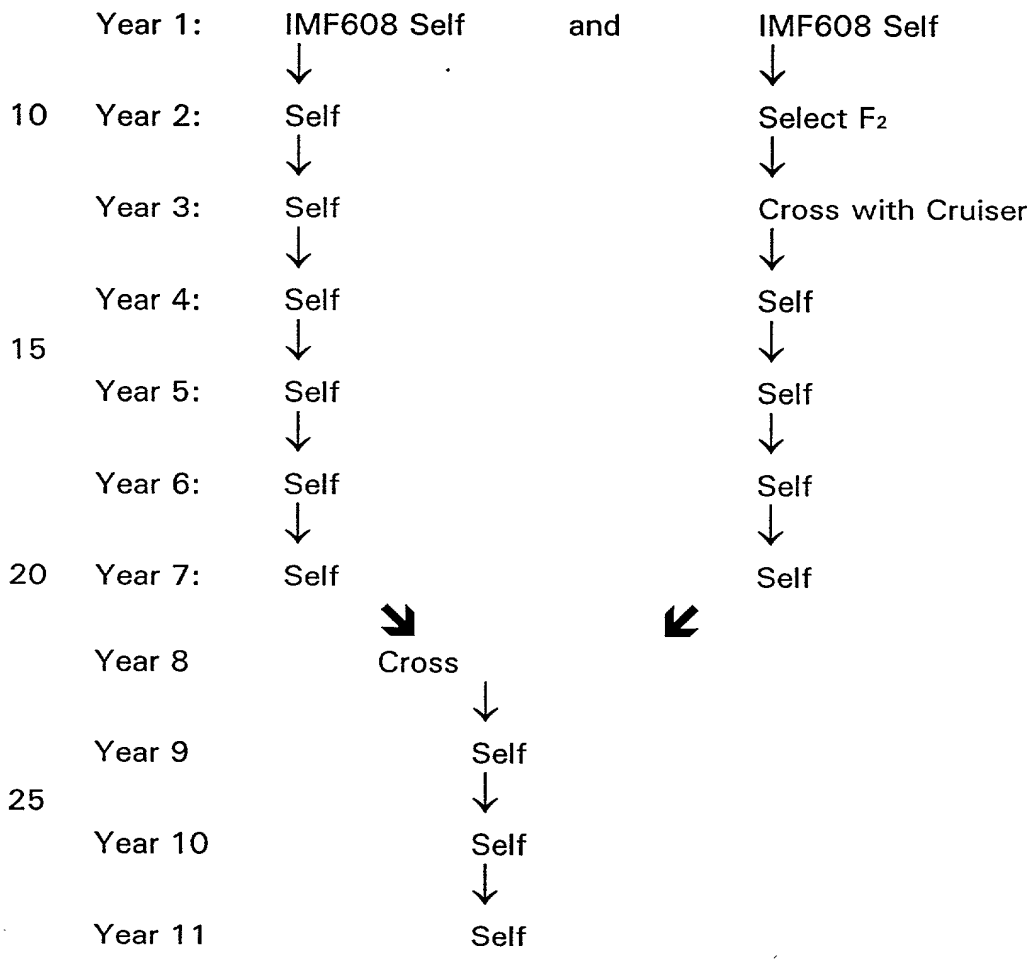
Derivation of: 4308-2; 4309-1; 4355-1; 4412-1; 4301; 4303; 4304; 4317; 4468; 4470; 4471



6. Inbred lines 4263-1; 4430-1; 4450-1 and 4450-2:

Inbred lines 4263-1; 4430-1; 4450-1 and 4450-2 were isolated following the pedigree chart outlined below using the techniques generally outlined above for inbred lines 393-2-19 and 393-2-47. Single plant selections were made for heat tolerance.

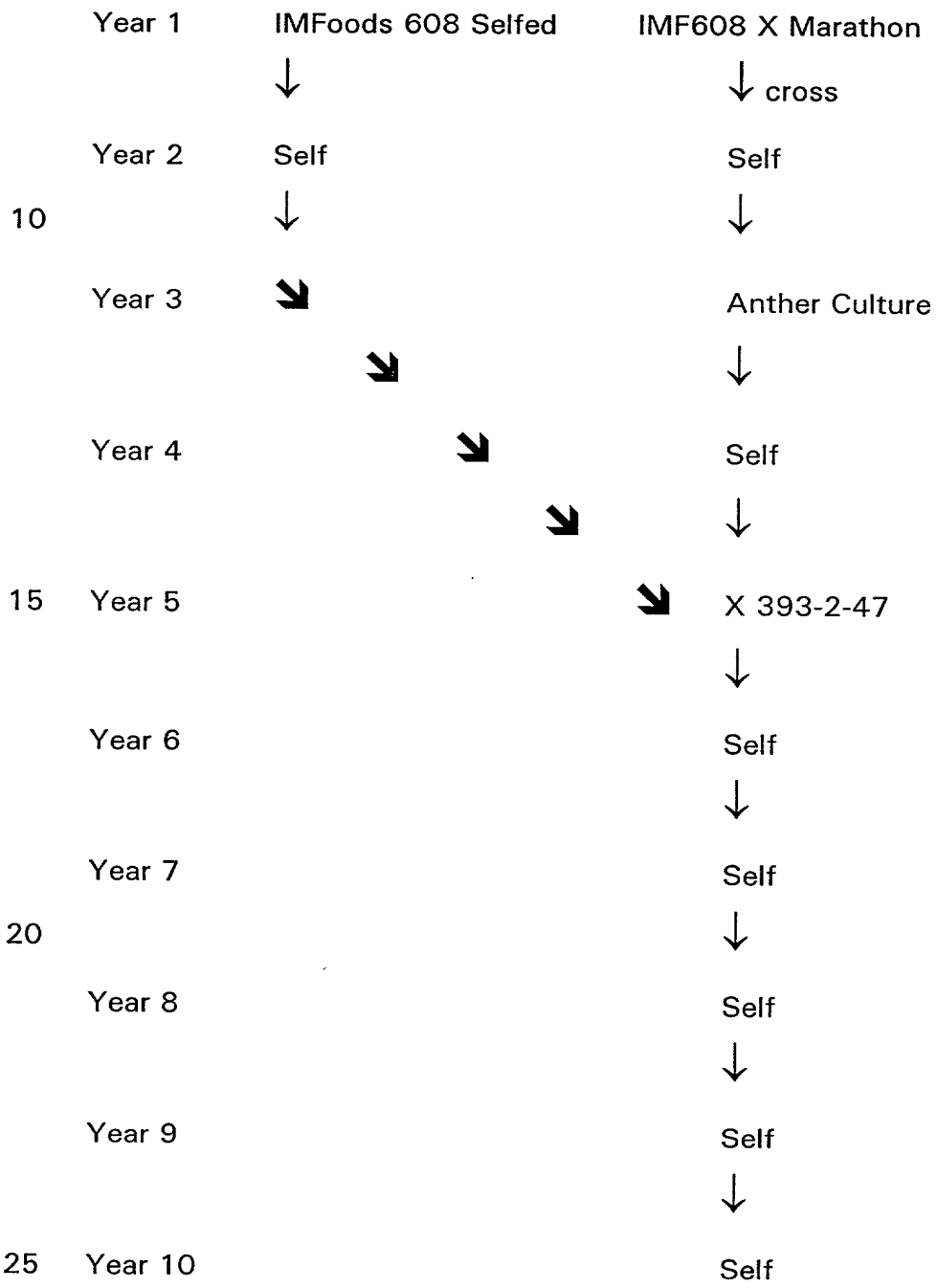
Derivation of: 4263-1; 4430-1; 4450-1; 4450-2



7. **Inbred line 4432-1**

Inbred line 4432-1 was isolated following the pedigree chart outlined below using the techniques generally outlined above for inbred lines 393-2-19 and 393-2-47. Single plant selections for heat tolerance were made.

Derivation of: 4432-1



8. **Inbred line 4267-1 (= "2192")**

Inbred line 4267-1 (= "2192") was isolated following the pedigree chart outlined below using the techniques generally outlined above for inbred line 393-2-19. Single plant selections were made for heat tolerance.

Derivation of: 4267-1 (= "2192")

Year 1	Cruiser X IMF608
	↓
Year 2	Self
	↓
Year 3	Self
	↓
Year 4	Self
	↓
Year 5	Self
	↓
Year 6	Self
	↓
Year 7	Open-pollinate

9. **Inbred line 7861**

Inbred line 7861 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7861

	Year 1	IMF 608	X	Southern Comet
			↓	
	Year 2		X	Green Belt
			↓	
	Year 3		X	Green Belt
			↓	
	Year 4		Self	
			↓	
	Year 5		Self	
			↓	
	Year 6		Self	
			↓	
	Year 7		Self	
			↓	
	Year 8		Self	

10. Inbred line 7864

Inbred line 7864 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7864

Year 1	Cruiser	X	Green Belt
		↓	
Year 2		X	Marathon
		↓	
Year 3		Self	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	

11. Inbred line 7865

Inbred line 7865 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7865

	Year 1	Synergene	6237	Self
		↓		
	Year 2	Self		
		↓		
	Year 3	Self		
		↓		
	Year 4	Self		
		↓		
	Year 5	Self		
		↓		
	Year 6	Self		
		↓		
	Year 7	Self		

12. **Inbred line 7881 and 1551**

Inbred lines 7881 and 1551 were isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7881 and 1551

Year 1	IMF 608	X	Green Belt
		↓	
Year 2		X	Green Belt
		↓	
Year 3		Self	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	
		↓	
Year 9		Self	

13. Inbred line 7887

Inbred line 7887 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7887

Year 1	IMF 608	Self	
		↓	
Year 2		X	Cruiser
		↓	
Year 3		Self	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	
		↓	
Year 9		Self	

14. Inbred line 7935

Inbred line 7935 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7935

Year 1	Seoul Star	X	IMF 608
		↓	
Year 2		Self	
		↓	
Year 3		Self	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	

15. Inbred line 8092

Inbred line 8092 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 8092

Year 1	Cruiser	X	Green Belt
		↓	
Year 2		X	Marathon
		↓	
Year 3		Self	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	
		↓	
Year 9		Self	

16. Inbred line 7883

Inbred line 7883 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7883

Year 1	IMF 608	Self
	↓	
Year 2	Self	
	↓	
Year 3	Self	
	↓	
Year 4	Self	
	↓	
Year 5	Self	

17. Inbred line 7914

Inbred line 7914 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7914

Year 1	Cruiser	X	Green Belt
		↓	
Year 2		X	IMF 608
		↓	
Year 3		X	Green Belt
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	
		↓	
Year 9		Self	

18. Inbred lines 7770 and 5580-2

Inbred lines 7770 and 5580-2were isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7770 and 5580-2

Year 1 Cruiser X Green Belt

↓

Year 2 X Marathon; IMF 608 Self

↘ ↙

Year 3 X ; IMF 608 X Arcadia

↘ ↙

Year 4 X

↓

Year 5 Self

↓

Year 6 Self

↓

Year 7 Self

↓

Year 8 Self

↓

Year 9 Self

↓

Year 10 Self

19. Inbred line 7778

Inbred line 7778 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7778

Year 1	IMF 608	Self
	↓	
Year 2	Self	
	↓	
Year 3	Self	
	↓	
Year 4	Self	
	↓	
Year 5	Self	
	↓	
Year 6	Self	
	↓	
Year 7	Self	
	↓	
Year 8	Self	

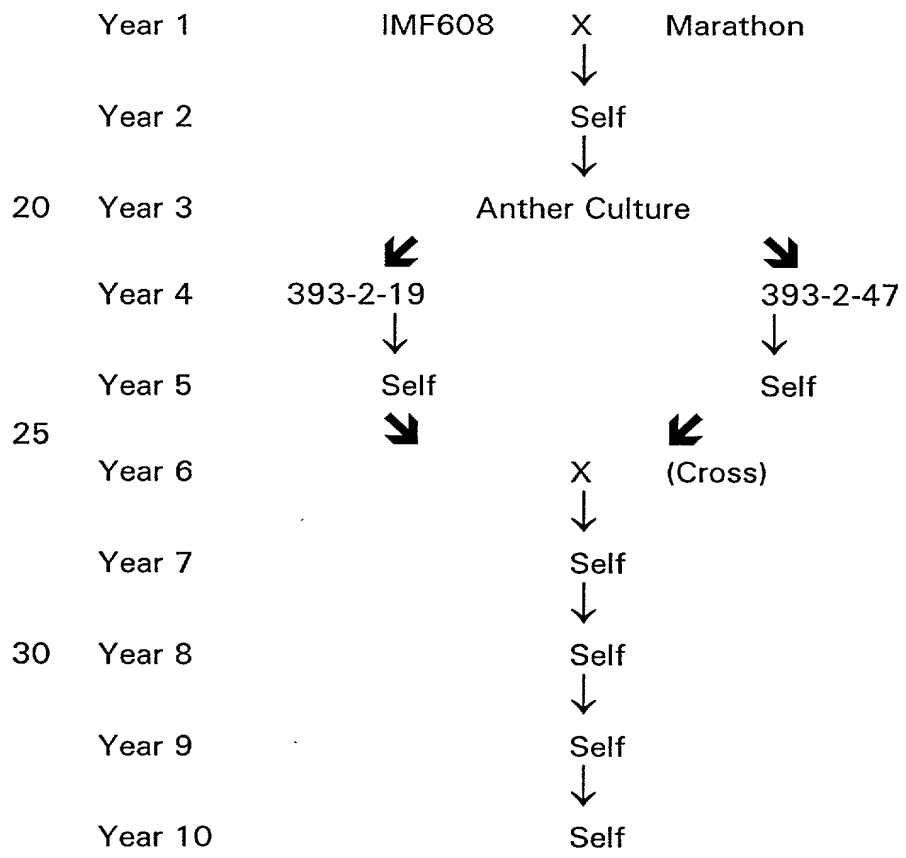
B. SELF INCOMPATIBLE LINES

Numerous heat tolerant self-incompatible ("female") lines were developed. For illustrative, but not limiting purposes, the breeding histories of the following self-incompatible lines are presented. Unless otherwise noted, single plant selections were made for heat tolerance.

1. Self-incompatible lines: 4201; 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1

Broccoli lines 4201; 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1 were isolated following the pedigree chart outlined below using the procedures generally outlined above for the isolation of 393-2-19.

Derivation of: 4201; 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1



2. Self Incompatible Line: 4415

Broccoli line 4415 was isolated following the pedigree chart outlined below using the procedures generally outlined above for 393-2-19.

Derivation of: 4415

Year 1	IMF608 X Green Belt
	↓
Year 2	X Green Belt
	↓
Year 3	Self
	↓
Year 4	Self
	↓
Year 5	Self
	↓
Year 6	Self
	↓
Year 7	Self
	↓
Year 8	Self
	↓
Year 9	Self
	↓
Year 10	Open-pollinate

3. **Self Incompatible Line: 4418**

Broccoli line 4418 was isolated following the pedigree chart outlined below using the procedures outlined above for 393-2-19.

Derivation of: 4418

Year 1	Green Belt X Cruiser		
	↓		
Year 2	X Marathon		Shogun X IMF608
	↘		↙
Year 3		Cross	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	

4. **Self Incompatible Line 4395-2**

Broccoli line 4395-2 was isolated following the pedigree chart outlined below using the procedures generally outlined above for 4935-2.

Derivation of: 4395-2

Year 1	Synergene 6236 Selfed
	↓
Year 2	Self
	↓
Year 3	Self
	↓
Year 4	Self
	↓
Year 5	Self
	↓
Year 6	Self
	↓
Year 7	Self
	↓
Year 8	Open pollinated
	↓
Year 9	Self

C. MALE LINES

Numerous heat tolerant "male" broccoli lines have been identified and shown stable and uniform. For illustrative but non-limiting purposes, the breeding histories of the M7007, M7009 and M7028 are provided as follows.

The "Cruiser" broccoli line was selected for initial crosses because it was a commercially available hybrid that showed a small degree of heat tolerance which was rated at approximately 5 and also had a nicely elevated head.

M7007

Year One	IM Hybrid No. 608 obtained from IM Foods, Incorporated, Gilroy, California, was self-pollinated.
Year Two	F2 of Hybrid No. 608 is crossed with Cruiser, which was obtained from Royal Sluis, a Dutch seed company.
Year Three	Heat tolerant single plant selection of the F2 Hybrid 608/Cruiser with heat tolerance equaling 8- was made.
Year Four	Heat tolerance equaling 7+ single plant selection gives [(No. 608) F2/Cruiser] F3.
Year Five	Eight selections are selfed and massed selected to give [(No. 608) F2/Cruiser] F4.
Year Six	Twelve selections are massed.
Year Seven	Fifteen selections are massed.
Year Eight	Five selections are massed selected and entered into a large isolation cage increase to give the finished line M7007.

M7009

Year One IM Hybrid No. 608 is self-pollinated.

Year Two F2 of Hybrid No. 608 is crossed with Cruiser.

Year Three Heat tolerant single plant selection of the F2 Hybrid 608/Cruiser with heat tolerance equaling 8- was made.

Year Four Heat tolerance equaling 7+ single plant selection gives [(No. 608) F2/Cruiser] F3.

Year Five Eight selections are selfed and massed to give [(No. 608) F2/Cruiser] F4.

Year Six Twelve selections are massed.

Year Seven Fifteen selections are massed.

Year Eight Five selections are massed selected and entered into a large isolation cage increase to give the finished line.

Year Nine Seed storage.

Year Ten Six selections are massed selected and entered into a large isolation cage increase to give finish line M7009.

M7028

Year Two F2 of Hybrid No. 608 is crossed with Cruiser.

Year Three Heat tolerant single plant selection of the F2 Hybrid 608/Cruiser with heat tolerance equaling 8- was made.

Year Four Made single plant selection. [(No. 608) F2/Cruiser] F3

Year Five Made single plant selection with heat tolerance equaling 7. [(No. 608) F2/Cruiser] F4

Year Six Made single plant selection with heat tolerance equaling 7. [(No. 608) F2/Cruiser] F5

Year Seven Selected five plants, massed selected and entered into a large isolation cage to give finished line M7028.

The male lines of this invention can be crossed with female lines (self-incompatible) to produce hybrid seed. The female lines

may or may not be heat tolerant. Encompassed within the scope of this invention are the hybrid seed produced from crossing the male lines of this invention with other broccoli lines of interest. Hybrid seed includes but is not limited to H7007, H7008, H7028.

HYBRID SEED PRODUCTION

For hybrid seed production of heat tolerant broccoli seed, two lines are selected for production. The lines are designated male or female, with the female being the recipient of the male line pollen. Either the male or female or both lines may be heat tolerant as defined by this invention. Broccoli plants flower with both the female and male parts and are capable of self-pollination. The line designated "female" is generally "self-incompatible," which means it will not accept its own pollen, a process developed in the plant by breeding. The line designated "male" is generally "self-compatible" and will accept its own pollen. Since self-incompatible lines will not accept their own pollen, but will out-cross with other broccoli pollen. Self-incompatible lines produce the commercially desired hybrid seed. The male line is the pollen provider to the female line. The cross of the self-compatible male line and the self-incompatible female line will produce a seed that is a hybrid.

Once a hybrid has been selected for seed production, a "nick" study is done. The nick study identifies the flowering period of the female, i.e. when it will start to flower and for how long it will flower. A nick study is also done for the male line and the two are compared. The nick study gives the data needed to determine if the female will require 1, 2, 3, or 4 male planting dates to cover its full flowering period.

Once the data from the nick study is obtained, seed of the female and the first male are planted in the greenhouse. The second male is planted in the greenhouse 7 – 10 days later, with the third

male planted another 7 – 10 days after that, and the final or 4th male planted within 10 more days. The female is seeded in the greenhouse at approximately 11,000 plants for each production acre and each male planting at 7,000 plants. Forty-five days from being planted in the greenhouse the female and first male are ready for transplanting in the field. The three remaining males are each transplanted into the field within 45 days of their individual greenhouse planting dates.

Field Production of Hybrid Seed

Field production of the hybrid seed is begun when all of the female plants and the first male plants from the greenhouse are transplanted into the field. Transplanting can be done by machine or by hand with large crews. The plants are placed into the soil on prepared listed beds that are on 40-inch centers (see Figure 2). The depth of the planting is generally 3 inches, but depends on the size of the transplant plug. Each plant is separated approximately eighteen inches apart going down the seed-line and each parallel seed-line on a single bed is twelve inches apart. The successive plantings of the second, third, and fourth male follow the female planting at approximately ten day intervals. An illustrative planting schedule is as follows:

October 15	female transplanting date
October 15	first male transplanting date
October 25	second male transplanting date
November 4	third male transplanting date
November 14	fourth male transplanting date

The dates are not fixed, but are an approximation for illustrative and non-limiting purposes.

Once all the plantings are accomplished, the field is watched for typical cultural problems found in all broccoli production, whether

for seed or vegetable. These problems include weeds, diseases, insect pests, irrigation, fertilization, and cultivation.

The singular difference for a seed production field as compared to a broccoli production field is the use of rogueing. Rogueing is simply the walking through and examination of a field and checking each plant for correctness to type. Any plant that does not fit the proper description for type is pulled and destroyed or "rogued." The rogueing starts within thirty days of the last male transplanted and continues until the field is at a market ready point, which is generally 100 days. Once the field is at market ready (market ready being the point where the heads are harvestable as a vegetable for sale) the seed production starts. Market ready heads are generally seen in the female and first male in late April to early May of the year following transplanting. The fully developed heads age and then bolt, which is the extension of individual flower stalks. The nick or timing between the male plants bolting and female plants bolting is now the crucial item watched. The female will only set hybrid seed if pollen is in constant and abundant supply from a male plant. The heads of both the male and female plant can be trimmed to accelerate or slow down the flowering to insure abundant male flowers are available as the female plant flowers. Pollen transfer from the male to the female is done by honeybees, which are commercially supplied. Each acre of seed production requires three to five hives of honeybees. The flowering stage will last sixty to eighty days.

The flowering period is followed by the maturation of the seed within seedpods. The maturation period of 40 to 60 days is checked by monitoring the seed development, as it goes from green and water filled, to the dough stage ending with the seed turning from green to brown in color. A judgment call is made, measuring the number of the mature seeds versus seeds yet completed. When the

majority of the seed is mature the female plants are cut by hand and laid in rows (windrowed) to dry down for combining. Ten to twenty days are needed for the plants to dry down.

Combining is a process, which entails the use of a large harvest machine that lifts the broccoli plants from the ground and grinds them for seed preparation. The plant material is cleaned away from the seed by screens and air, leaving only seed. Combining is the initiation of the seed re-conditioning process. Once combined or harvested, the seed is sent to a mill, which further cleans the seed, separates the clean seed by size and weight within a size. All testing for purity, disease, germination, and percent hybridity is done on the clean, sized, and weighted seed. If the seed passes the testing it is canned for sale.

The above method describes the seed production methods for the specific hybrids H7007, H7009, H7022, H2061, H2088, H7021R and H7028 and generally is the method used for all other hybrid broccoli seed production. Hybrid seeds H7007, H7009 H7022, H2061, H2088, H7021R and H70028 were produced by crossing corresponding male lines with 393-2-19 as follows:

H7007 = 393-2-19 X male 7007

H7009 = 393-2-19 X male 7009

H7022 = 393-2-19 X male 5580-2 (same derivation as 7770)

H2061 = 393-2-19 X male 1551 (same derivation as 7881)

H2088 = 393-2-19 male 7009

H7021R = 5580 (same derivation as 7770) X 393-2-19

H7028 = 393-2-19 X male 7028

COMPARATIVE STUDIES

Several studies have been performed to compare and contrast the broccoli lines of this invention with commercially available broccoli lines.

Comparative Analysis Study #1

In study #1, the following broccoli lines were analyzed: Hybrid 7007, hybrid 7008, hybrid 7022, hybrid 7028, male 7007, male 7009, male 7022, male 7028, hybrid 393-2-19, hybrid 393-2-47, Marathon and Pinnacle. Marathon and Pinnacle are commercially available broccoli hybrids. Hybrid 7022 resulted from a cross between 393-12-19 and 5580-2 (393-2-19/5580-2). As indicated above, 5580 is the same derivative as 7770.

Broccoli seeds were sown in the greenhouse. Broccoli seedlings were transplanted to the field on August 8. Daily high and low temperature measurements during the course of study #1 are presented in Table 1. Note that the growing temperatures for study #1 were generally quite warm.

In study #1, the days from direct seeding to 50% harvest; days from transplanting to 50% harvest and the length of the harvest period are shown in Table 2.1. The results indicate that the broccoli lines of this invention have a significantly longer harvest period than the commercially available hybrids Marathon and Pinnacle. A longer period in which the head remains available for harvest offers growers greater flexibility in harvesting and therefore greatly reduces costs. The harvest "holding" ability is due, in part, to heat tolerance.

Table 2.2 shows data summarizing various characteristics of the broccoli plants at harvest. Tables 2.3A and 2.3B show data regarding the characteristics of the outer leaves at harvest. The data indicate that both Pinnacle and Marathon were gray-green in foliage color, which is demonstratively different and less commercially acceptable than the blue green foliage of the heat tolerant lines of the invention.

Table 2.4A-2.4D show characteristics of the broccoli heads at market maturity. Table 2.5 shows flower color.

Table 2.6 shows resistance to various environmental conditions, undesirable characteristics of broccoli and diseases. Of particular importance is that the commercially available varieties Marathon and Pinnacle are much more susceptible to downy mildew virus as compared to the broccoli lines of the invention.

Table 2.7 shows heat tolerance data. Of particular relevance is the low heat tolerance of the commercially available varieties Marathon and Pinnacle as compared to the broccoli lines of this invention.

TABLE 1

Temperature Data for Study #1

Date	Temperature (°F)		
	Max	min	Average
07/03	79	47	62
07/04	84	48	63
07/05	75	52	60
07/06	75	52	62
07/07	75	52	61
07/08	71	48	59
07/09	63	54	58
07/10	70	54	59
07/11	73	53	59
07/12	74	52	59
07/13	85	53	65
07/14	87	54	69
07/15	82	50	63
07/16	72	32	62
07/17	76	56	64
07/18	83	58	68
07/19	89	52	69
07/20	83	53	67
07/21	88	53	71
07/22	100	55	78
07/23	99	59	77
07/24	88	56	69
07/25	95	54	72
07/26	81	58	70
07/27	76	55	63
07/28	78	55	62
07/29	75	56	62
07/30	72	56	61
07/31	72	57	62
08/01	82	57	65
08/02	83	56	65
08/03	88	54	68
08/04	83	56	66
08/05	77	56	64
08/06	74	58	64
08/07	79	59	66
08/08	90	56	71
08/09	98	59	74
08/10	109	60	81
08/11	100	61	78
08/12	91	58	70

TABLE 1

Temperature Data for Study #1

Date	Temperature (°F)		
	Max	min	Average
08/13	83	56	66
08/14	84	52	64
08/15	81	50	62
08/16	86	50	66
08/17	92	53	71
08/18	98	58	75
08/19	97	60	75
08/20	92	57	71
08/21	89	58	68
08/22	74	54	61
08/23	74	53	61
08/24	72	51	61
08/25	69	53	60
08/26	71	52	60
08/27	77	54	63
08/28	81	50	63
08/29	86	51	67
08/30	87	50	67
08/31	83	51	64
09/01	69	54	59
09/02	81	51	61
09/03	91	48	64
09/04	95	52	71
09/05	94	46	72
09/06	95	57	71
09/07	91	55	69
09/08	93	54	71
09/09	98	54	69
09/10	91	58	71
09/11	83	61	69
09/12	89	64	73
09/13	95	68	77
09/14	93	64	75
09/15	77	56	64
09/16	68	56	60
09/17	83	32	70
09/18	85	52	67
09/19	87	55	69
09/20	88	57	68
09/21	76	55	62
09/22	73	53	61

Table 2: Comparative Analysis (Study #1)

2.1. Region of Adaption/Maturity Main Crop at 50% Harvest

#	I.D.	Region of Adaption	Days from Direct Seeding to 50% Harvest	Days from Transplanting to 50% Harvest	Length of Harvest Period in Days
1	Hybrid 7007	Most regions	137	84	7
2	Hybrid 7008	Most regions	137	87	6
3	Hybrid 7022	Most regions	127	77	6
4	Hybrid 7028	Most regions	136	86	6
5	Male 7007	Southwest	135	85	3
6	Male 7009	Southwest	135	85	4
7	Male 7022	Southwest	123	73	5
8	Male 7028	Southwest	138	88	5
9	Hybrid 393-2-19	Most regions	137	87	8
10	Hybrid 393-2-47	Most regions	133	83	6
11	Marathon	Most regions	134	84	4
12	Pinnacle	Southwest	123	73	2

2.2. <u>Study #1</u>
<u>Plant (At Harvest)</u>

#	I.D.	Plant Height (cm)	Head Height (cm)	Plant Branches	Plant Habit	Market Class	Lifecycle	Variety Type
1	Hybrid 7007	76.5	57.5	Few	Intermediate	Fresh Market/ Processing	Annual	First generation hybrid
2	Hybrid 7008	-	-	-	-	-	-	-
3	Hybrid 7022	72	51.5	Few	Spreading	Fresh Market/ Processing	Annual	First generation hybrid
4	Hybrid 7028	82.5	57.5	Few	Intermediate	Fresh Market/ Processing	Annual	First generation hybrid
5	Male 7007	92	65	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred
6	Male 7009	92	76	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred
7	Male 7022	58	35	Few	Compact	Fresh Market/ Processing	Annual	Inbred
8	Male 7028	74.5	51	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred

2.2. Study #1
Plant (At Harvest)

#	I.D.	Plant Height (cm)	Head Height (cm)	Plant Branches	Plant Habit	Market Class	Lifecycle	Variety Type
9	Inbred 393-2-19	62	45.5	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred
10	Inbred 393-2-47	60	48.5	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred
11	Marathon	86.5	56.5	Medium	Spreading	Fresh Market/ Processing	Annual	First generation hybrid
12	Pinnacle	88.5	61.5	Few	Intermediate	Fresh Market/ Processing	Annual	First generation hybrid

2.3A. Study #1
Outer Leaves (At Harvest)

#	I.D.	# Leaves Per Plant	Leaf Width (cm)	Leaf Length (cm)	Petiole Length (cm)	Leaf Attachment	Wax Presence	Foliage Color
1	Hybrid 7007	29	20	52.5	22	Petiolate	Strong	Blue-green
2	Hybrid 7008	-	-	-	-	-	-	-
3	Hybrid 7022	18	14	41	17.5	Petiolate	Strong	Blue-green
4	Hybrid 7028	25	17.5	53.5	19.5	Petiolate	Strong	Blue-green
5	Male 7007	30	16.5	40.5	13.5	Petiolate	Strong	Blue-green
6	Male 7009	26	15.5	47	18	Petiolate	Strong	Blue-green
7	Male 7022	21	23.5	48	17.5	Petiolate	Strong	Blue-green
8	Male 7028	34	15.5	42.5	19.	Petiolate	Strong	Blue-green
9	Inbred 393-2-19	23	14	36	11.5	Petiolate	Strong	Blue-green
10	Inbred 393-2-47	24	16.5	40	17.5	Petiolate	Strong	Blue-green
11	Marathon	50	15.5	50	22	Petiolate	Intermediate	Grey-green
12	Pinnacle	27	16	46.5	21	Petiolate	Intermediate	Grey-green

2.3B. Study #1

Outer Leaves (At Harvest)

#	I.D.	Leaf Shape	Leaf Base	Leaf Apex	Leaf Margins	Leaf Veins	Midrib	Blistering	Attitude	Leaf tip Torsion	Upper Side of Leaf Profile
1	Hybrid 7007	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Semi-erect	Weak	Concave
2	Hybrid 7008	-	-	-	-	-	-	-	-	-	-
3	Hybrid 7022	Narrow elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Semi-erect	None	Planar
4	Hybrid 7028	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Semi-erect	None	Concave
5	Male 7007	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Erect	Weak	Concave
6	Male 7009	Elliptic	Pointed	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Erect	Intermediate	Planar
7	Male 7022	Broad elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Not raised	None	Horizontal/ Semi-erect	Weak	Concave

2.3B. Study #1
Outer Leaves (At Harvest)

#	I.D.	Leaf Shape	Leaf Base	Leaf Apex	Leaf Margins	Leaf Veins	Midrib	Blistering	Attitude	Leaf tip Torsion	Upper Side of Leaf Profile
8	Male 7028	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Erect	None	Concave
9	Inbred 393-2-19	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Erect	None	Concave
10	Inbred 393-2-47	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Semi-erect	None	Concave
11	Marathon	Narrow elliptic	Blunt	Blunt	Very wavy	Inter-mediate	Slightly raised	None	Horizontal/ Semi-erect	None	Concave
12	Pinnacle	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Horizontal	None	Concave

2.4A. Study #1
Head (At Market Maturity)

#	I.D.	Head Diameter (cm)	Head Depth (cm)	Head Weight (gm)	Head Color	Head Shape
1	Hybrid 7007	17	13.5	446.3	Blue/Green	Transverse broad elliptic
2	Hybrid 7008	-	-	352	-	-
3	Hybrid 7022	15	10.5	377.2	Blue/Green	Transverse narrow elliptic
4	Hybrid 7028	15	11.5	364.4	Blue/Green	Transverse broad elliptic
5	Male 7007	10	8	93.7	Blue/Green	Circular
6	Male 7009	10	8.5	126.2	Blue/Green	Transverse broad elliptic
7	Male 7022	15	10	289.5	Blue/Green	Transverse narrow elliptic
8	Male 7028	11	9.5	165.9	Blue/Green	Circular
9	Inbred 393-2-19	13	10	325.7	Blue/Green	Transverse elliptic
10	Inbred 393-2-47	11.5	9	194.7	Blue/purple	Transverse broad elliptic
11	Marathon	14.5	12.5	300	Medium green	Transverse elliptic
12	Pinnacle	14	9.5	274.6	Medium green	Transverse elliptic
13	Male 7008	-	-	150	-	-

2.4B. Study #1
Head (At Market Maturity)

#	I.D.	Dome Shape	Head Size	Compactness	Surface Knobbling	Bead Size	Flower Buds
1	Hybrid 7007	Semi-domed	Large	Short pedicels (tight)	Fine	Medium	Even in size
2	Hybrid 7008	-	-	-	-	-	-
3	Hybrid 7022	Very Deeply domed	Large	Short pedicels (tight)	Fine	Medium	Even in size
4	Hybrid 7028	Semi-domed	Medium	Short pedicels (tight)	Fine	Medium	Even in size
5	Male 7007	Domed	Small	Short pedicels (tight)	Fine	Large	Even in size
6	Male 7009	Semi-domed	Small	Medium pedicels	Fine	Large	Even in size
7	Male 7022	Very deeply domed	Large	Short pedicels (tight)	Fine	Medium	Even in size
8	Male 7028	Domed	Small	Medium pedicels	Fine	Medium	Even in size
9	Inbred 393-2-19	Deep domed	Medium	Short pedicels (tight)	Fine	Small	Even in size
10	Inbred 393-2-47	Semi-domed	Medium	Short pedicels (tight)	Fine	Medium	Even in size
11	Marathon	Deep Domed	Medium	Short pedicels (tight)	Medium	Small	Even in size
12	Pinnacle	Deep Domed	Medium	Medium pedicels	Large	Small	Uneven in size

2.4C. Study #1

Head (At Market Maturity) Anthocyanin Coloration

#	I.D.	Leaf Axils	Leaf Veins	Leaf Blade	Entire Plant	Leaf Petiole
1	Hybrid 7007	Absent	Absent	Absent	Absent	Absent
2	Hybrid 7008	-	-	-	-	-
3	Hybrid 7022	Absent	Absent	Absent	Absent	Absent
4	Hybrid 7028	Absent	Absent	Absent	Absent	Absent
5	Male 7007	Absent	Absent	Absent	Absent	Absent
6	Male 7009	Absent	Absent	Absent	Absent	Absent
7	Male 7022	Absent	Absent	Absent	Absent	Absent
8	Male 7028	Absent	Absent	Absent	Absent	Absent
9	Inbred 393-2-19	Absent	Absent	Absent	Absent	Absent
10	Inbred 393-2-47	Slight Pressure	Absent	Absent	Absent	Absent
11	Marathon	Absent	Absent	Absent	Absent	Absent
12	Pinnacle	Absent	Absent	Absent	Absent	Absent

2.4D. Study #1
Head (At Market Maturity)

#	I.D.	Color of Head Leaves	Secondary Heads	Prominence of Secondary Heads	Number of Secondary Heads
1	Hybrid 7007	Green	Completely Absent	Weak	0
2	Hybrid 7008	-	-	-	-
3	Hybrid 7022	Green	Completely Absent	Weak	0
4	Hybrid 7028	Green	Basal	Weak	3
5	Male 7007	Green	Completely Absent	Weak	0
6	Male 7009	Green	Basal	Weak	4
7	Male 7022	Green	Completely Absent	Weak	0
8	Male 7028	Green	Completely Absent	Weak	0
9	Inbred 393-2-19	Green	Completely Absent	Weak	0
10	Inbred 393-2-47	Green	Completely Absent	Weak	0
11	Marathon	Green	Auxiliary along entire main stem up to main head	Weak	3
12	Pinnacle	Green	Basal	Weak	1

2.5. Study #1
Flower Color

#	I.D.	Flower Color	Flower Stalk Color
1	Hybrid 7007	Yellow	Green
2	Hybrid 7008	-	-
3	Hybrid 7022	Yellow	Green
4	Hybrid 7028	Yellow	-
5	Male 7007	Yellow	-
6	Male 7009	Yellow	-
7	Male 7022	Yellow	Green
8	Male 7028	Yellow	-
9	Inbred 393-2-19	Yellow	Green
10	Inbred 393-2-47	Yellow	Green
11	Marathon	Yellow	Green
12	Pinnacle	Yellow	Green

5

2.6. <u>Study #1</u>
<u>Resistance*</u>

#	I.D.	Downey Mildew	Buttoning	Blindness	Bolting	Brown Beads	Drought	Cold	Hollow stem	Riceyness	Whiptail
1	Hybrid 7007	9	8	8	5	9	7	5	8	9	9
2	Hybrid 7008	-	-	-	-	-	-	-	-	-	-
3	Hybrid 7022	9	8	8	4	8	7	3	8	9	9
4	Hybrid 7028	9	8	8	4	9	7	4	8	9	9
5	Male 7007	9	8	8	3	9	8	3	9	9	9
6	Male 7009	9	8	8	5	9	8	3	9	9	9
7	Male 7022	-	8	8	6	8	8	3	8	9	9
8	Male 7028	9	8	8	5	9	8	3	8	9	9
9	Inbred 393-2-19	9	8	8	6	9	8	6	9	9	9
10	Inbred 393-2-47	9	8	8	5	9	8	5	9	9	9
11	Marathon	3	8	8	7	8	7	7	8	9	9
12	Pinnacle	3	8	8	6	5	5	4	8	5	9

* **1 = Most susceptible**
5 = Intermediate
9 = Most resistant

2.7. Study #1
Heat Tolerance*

#	I.D.	Heat Tolerance*
1	Hybrid 7007	9
2	Hybrid 7008	-
3	Hybrid 7022	8
4	Hybrid 7028	9
5	Male 7007	9
6	Male 7009	8
7	Male 7022	7
8	Male 7028	8
9	Inbred 393-2-19	8
10	Inbred 393-2-47	8
11	Marathon	2
12	Pinnacle	4

* **1 = Most susceptible**
5 = Intermediate
9 = Most tolerant

Comparative Analysis Study #2

In a second study (Study #2) various broccoli lines were analyzed and characterized for heat tolerance. Daily high and low temperature measurements for study #2 are presented in Table 3. As in study #2, the daily temperatures were generally quite warm and on some days hot.

In study #2, the following broccoli lines were analyzed: Hybrid 7007, Hybrid 7009, Hybrid 7028, Male 7007, Male 7009, Male 7028, Inbred 393-2-19, Inbred 393-2-47, Marathon, Pinnacle, 98-2061, 98-2088, Inbred 393-2-32 and 4267-1. 98-2061 results from a cross between 393-2-19 and 1551 (393-2-19/1551). As indicated above, 1551 is the same derivative as 7881. The line 98-2088 results from across between 393-2-19 and M7009 (393-2-19/1551). Line 2192 is derived from the same line as 4267-1.

In study #2, broccoli seeds were sown in the greenhouse on April 27. Broccoli seedlings were transferred to the field on June 13.

The comparative data collected in study #2 are shown in Table 4.

Table 4.1 shows the length of the harvest period, the plant and head height at harvest, the type of plant branches and the plant habit at harvest. Of particular relevance is that the broccoli plants of this invention have a significantly longer harvest period than the commercially available hybrids Marathon and Pinnacle. A longer harvest period offers growers greater flexibility in harvesting and therefore greatly reduces costs.

Tables 4.2A-4.2C show characteristics of outer leaves at harvest. Tables 4.3A-4.3B and 4.4A show characteristics of the harvested broccoli heads. Table 4.5 shows heat tolerance data.

Of particular relevance is the data in Table 4.5, which shows

that the broccoli plants of this invention are heat tolerant whereas the commercially available varieties are not.

TABLE 3

Temperature Data for Study #2

Date	Temperature (°F)		
	Max	min	Average
08/08	75	57	63
08/09	81	60	66
08/10	77	58	65
08/11	78	57	65
08/12	79	53	63
08/13	78	55	63
08/14	83	54	64
08/15	77	56	62
08/16	73	56	63
08/17	89	53	63
08/18	83	54	66
08/19	82	59	69
08/20	77	59	66
08/21	87	59	69
08/22	85	56	70
08/23	83	59	69
08/24	82	62	70
08/25	83	57	68
08/26	83	57	68
08/27	83	51	70
08/28	83	56	69
08/29	84	59	69
08/30	82	55	67
08/31	83	59	70
09/01	84	59	70
09/02	81	56	68
09/03	85	59	69
09/04	95	59	73
09/05	87	58	70
09/06	80	55	65
09/07	88	53	66
09/08	86	59	69
09/09	82	55	66
09/10	80	54	66
09/11	79	58	67
09/12	78	54	65
09/13	78	53	63
09/14	79	54	65
09/15	80	56	66

TABLE 3

Temperature Data for Study #2

Date	Temperature (°F)		
	Max	min	Average
09/16	85	51	68
09/17	79	54	66
09/18	78	54	64
09/19	82	48	63
09/20	88	51	68
09/21	89	51	66
09/22	87	49	66
09/23	102	54	75
09/24	97	59	74
09/25	87	61	72
09/26	80	57	67
09/27	87	52	68
09/28	95	52	73
09/29	90	59	69
09/30	94	54	65
10/01	74	56	63
10/02	76	57	64
10/03	83	51	66
10/04	81	51	65
10/05	83	50	64
10/06	69	49	59
10/07	70	46	57
10/08	72	43	57
10/09	65	52	60
10/10	62	44	53
10/11	68	44	55
10/12	74	44	57
10/13	83	42	61
10/14	89	44	65
10/15	95	49	68
10/16	96	50	68
10/17	87	50	64
10/18	81	46	59
10/19	64	49	56
10/20	70	49	57
10/21	74	45	55
10/22	70	45	54
10/23	68	49	57
10/24	72	42	55
10/25	75	38	56

TABLE 3

Temperature Data for Study #2

Date	Temperature (°F)		
	Max	min	Average
10/26	79	40	57
10/27	75	42	56

TABLE 4 COMPARATIVE ANALYSIS Study #2

4.1. Maturity: Main Crop at 50% Harvest/Plant At Harvest

#	I.D.	Length of Harvest Period (Days)	Plant Height (inches)	Head Height (inches)	Plant Branches	Plant Habit
1	Hybrid 7007	5	30	22	Medium	Spreading
2	Hybrid 7009	5	28¼	22	Medium	Spreading
3	Hybrid 7028	4	25 5/8	17	Medium	Spreading
4	Male 7007	3	36	33	Medium	Spreading
5	Male 7009	4	37	25½	Many	Spreading
6	Male 7028	-	31	23½	Medium	Spreading
7	Inbred 393-2-19	6	27	20	Medium	Spreading
8	Inbred 393-2-47	4.5	26	20	Few	Spreading
9	Marathon	1	30.5	20	Many	Intermediate
10	Pinnacle	1	30	26¾	Medium	Intermediate
11	98-2061	5	26	18	Medium	Intermediate
12	98-2088	4	29¼	23¾	Medium	Intermediate
13	Inbred 393-2-32	6	27	18¼	Medium	Intermediate
14	98-2192	6	28	16	Medium	Spreading

4.2A. <u>Study #2</u>

Outer Leaves (At Harvest)

#	I.D.	# Leaves Per Plant	Leaf Width (inches)	Leaf Length (inches)	Petiole Length (inches)	Length/Width Ratio
1	Hybrid 7007	23	8	18¼	5	2:1
2	Hybrid 7009	26	8	17	5	2:1
3	Hybrid 7028	22	6½	16	4¾	2:1
4	Male 7007	24	10	19	7	2:1
5	Male 7009	32	9	21½	6¾	2:1
6	Male 7028	18	10½	22½	6½	2:1
7	Inbred 393-2-19	21	7	16½	5½	2:1
8	Inbred 393-2-47	17	5¾	11¾	3½	2:1
9	Marathon	32	7	18	8¼	2:1
10	Pinnacle	25	5 5/8	14½	7¼	2:1
11	98-2061	19	6½	16½	6¼	2:1
12	98-2088	28	7¼	14¾	3½	2:1
13	Inbred 393-2-32	21	6½	19	8¼	2:1
14	98-2192	28	8¼	18½	7¾	2:1

4.2B. Study #2
Outer Leaves (At Harvest)

#	I.D.	Leaf Attachment	Wax Presence	Foliage Color	Leaf Shape	Leaf Base
1	Hybrid 7007	Petiolate	Strong	Medium green	Elliptic	Pointed
2	Hybrid 7009	Petiolate	Strong	Medium green	Elliptic	Blunt
3	Hybrid 7028	Petiolate	Strong	Medium green	Elliptic	Blunt
4	Male 7007	Petiolate	Strong	Medium green	Elliptic	Blunt
5	Male 7009	Petiolate	Strong	Medium green	Broad elliptic	Blunt/pointed
6	Male 7028	Petiolate	Strong	Medium green	Broad elliptic	Blunt/pointed
7	Inbred 393-2-19	Petiolate	Strong	Medium green	Elliptic	Blunt
8	Inbred 393-2-47	Petiolate	Strong	Medium green	Elliptic	Blunt
9	Marathon	Petiolate	Strong	Blue-green	Elliptic	Blunt
10	Pinnacle	Petiolate	Strong	Medium green	Narrow elliptic	Blunt
11	98-2061	Petiolate	Strong	Dark green	Elliptic	Blunt
12	98-2088	Petiolate	Strong	Medium green	Narrow elliptic	Blunt
13	Inbred 393-2-32	Petiolate	Strong	Medium green	Elliptic	Blunt
14	98-2192	Petiolate	Strong	Dark green	Elliptic	Blunt

4.2C. Study #2

Outer Leaves (At Harvest)

#	I.D.	Leaf Apex	Leaf Margins	Leaf Veins	Attitude	Torsion	Profile
1	Hybrid 7007	Blunt	Slightly wavy	Intermediate	Horizontal	Weak	Planar
2	Hybrid 7009	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Weak	Planar
3	Hybrid 7028	Blunt	Slightly wavy	Thin	Semi-erect/erect	Weak	Planar
4	Male 7007	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Weak	Planar
5	Male 7009	Blunt	Very wavy	Intermediate	Semi-erect/erect	Weak	Planar/convex
6	Male 7028	Blunt	Slightly wavy	Thick	Horizontal/semi-erect	Weak	Planar
7	Inbred 393-2-19	Blunt	Slightly wavy	Intermediate	Semi-erect	Weak	Concave
8	Inbred 393-2-47	Blunt	Slightly wavy	Intermediate	Horizontal/semi-erect	Weak	Concave/planar
9	Marathon	Blunt	Very wavy	Intermediate	Horizontal	Intermediate	Concave
10	Pinnacle	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Intermediate	Convex
11	98-2061	Blunt	Slightly wavy	Thin	Semi-erect	Weak	Planar
12	98-2088	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Weak	Concave
13	Inbred 393-2-32	Blunt	Slightly wavy	Intermediate	Horizontal	Weak	Planar
14	98-2192	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Weak	Planar/convex

4.3A. Study #2

Head (At Market Maturity)

#	I.D.	Head Diameter (inches)	Head Depth (inches)	Head Weight (gm)	Color	Head Shape
1	Hybrid 7007	10	5 ¼	904.9	Blue/green	Transverse elliptic
2	Hybrid 7009	7	3 ¼	306.4	Purple/ blue/green	Transverse elliptic
3	Hybrid 7028	4 ¾	3	117	Blue/green	Transverse elliptic
4	Male 7007	4 ¼	2 ½	85.6	Medium green	Circular
5	Male 7009	3 ¾	2 ¼	103.6	Dark green/ Blue/green	Transverse broad elliptic
6	Male 7028	6	3 ½	450.3	Light purple/ dark green	Transverse elliptic
7	Inbred 393-2-19	5	3	176.3	Blue/green purple	Transverse elliptic narrow
8	Inbred 393-2-47	4 ¾	2 ¾	136.4	Light green/ purple	Transverse elliptic
9	Marathon	4 ½	2 ¼	313.1	Yellow	Transverse elliptic
10	Pinnacle	6 ¼	4 ¾	336.3	Blue/green	Transverse elliptic
11	98-2061	5 ½	3	184.1	Blue/green	Transverse broad elliptic
12	98-2088	5 ¾	3 ½	184.1	Blue/green/ purple	Transverse elliptic
13	Inbred 393-2-32	3 ¼	2 ½	67.2	Medium green/ blue/green	Transverse broad elliptic
14	98-2192	5 ½	3	226.0	Blue/green	Transverse elliptic

4.3B. Study #2
Head (At Market Maturity)

#	I.D.	Dome Shape	Head Size	Compactness	Surface Knobbling	Beads Size	Flower Buds
1	Hybrid 7007	Domed	Large	Medium pedicels	Medium	Medium/large	Even in size
2	Hybrid 7009	Deep-domed	Medium	Medium pedicels	Medium	Medium	Even in size
3	Hybrid 7028	Semi-domed	Medium	Short pedicels	Medium	Small	Even in size
4	Male 7007	Domed	Small	Medium pedicels	Fine	Medium	Even in size
5	Male 7009	Deep-domed	Small	Short pedicels	Fine	Small	Even in size
6	Male 7028	Deep-domed	Medium	Short pedicels	Medium	Small	Even in size
7	Inbred 393-2-19	Deep-domed	Medium	Short pedicels	Medium	Small	Even in size
8	Inbred 393-2-47	Domed	Medium	Short pedicels	Medium	Small	Even in size
9	Marathon	Domed	Small	Short pedicels	Medium	Small	Uneven in size
10	Pinnacle	Domed	Medium	Long pedicels	Coarse	Large	Uneven in size
11	98-2061	Semi-domed	Medium	Short pedicels	Medium	Small	Even in size
12	98-2088	Semi-domed	Medium	Medium pedicels	Medium	Medium	Even in size
13	Inbred 393-2-32	Semi-domed	Small	Short pedicels	Fine	Small	Even in size
14	98-2192	Deep-domed	Medium	Short pedicels	Medium	Small	Even in size

4.4A. Study #2
Head (At Market Maturity)

#	I.D.	Color of Head Leaves	Secondary Heads	Prominence of Secondary Heads	# of Secondary Heads
1	Hybrid 7007	---	---	---	---
2	Hybrid 7009	---	---	---	---
3	Hybrid 7028	---	Basal	Weak	1
4	Male 7007	---	Basal	Weak	0
5	Male 7009	Green	---	---	---
6	Male 7028	---	---	---	---
7	Inbred 393-2-19	---	---	---	---
8	Inbred 393-2-47	---	---	---	---
9	Marathon	---	Completely absent	Weak	0
10	Pinnacle	---	Basal	Weak	3
11	98-2061		Basal	Weak	1
12	98-2088		Basal	Intermediate	4
13	Inbred 393-2-32		---	---	---
14	98-2192		Combination	Intermediate	3

4.5. Study #2
Heat Tolerance

#	I.D.	Heat Tolerance*
1	Hybrid 7007	7
2	Hybrid 7009	8
3	Hybrid 7028	7
4	Male 7007	---
5	Male 7009	7/8
6	Male 7028	---
7	Inbred 393-2-19	5/6
8	Inbred 393-2-47	5/6
9	Marathon	2
10	Pinnacle	1
11	98-2061	7/8
12	98-2088	7
13	Inbred 393-2-32	---
14	98-2192	8/9

* **1 = Most susceptible**
5 = Intermediate
9 = Most tolerant

Comparative Analysis Study #3

In a third study (Study #3) various broccoli lines were analyzed and characterized for heat tolerance. Lines tested included H7009, H7007, H7028, H7010 H7021R, Marathon, Pinnacle, etc. Lines which include a backslash (/) between the two lines represent a cross between the two lines. The second line on the right side of the backslash, is the "male" line in the cross. The "male" line in the cross is the source of the pollen in the cross. For example 393-2-19/7770 represents a cross between 393-2-19 and 7770 wherein 7770 was the source of the pollen and 393-2-19 was the recipient of the pollen. Single plant selections were made of the crosses. The resulting seed was then selfed. The data presented is summary data based upon an entire row of plants.

As indicated above, H7021R results from a cross between 5580 and 393-2-19 (5580/393-2-19)

Daily high and low temperature measurements for study #3 are presented in Table 5. As in studies #'s 1 and 2, the growth temperatures during study #3 were generally quite warm and sometimes hot.

Various broccoli lines were analyzed for heat tolerance. The heat tolerance data is presented in Table 6.

The commercial hybrids (Marathon, Pinnacle, Premium Crop, Patriot, Laguna, Monte Cristo, Greenbelt, Everest, CMS Liberty, and Landmark) averaged a score of 2.83 for heat tolerance. The new heat tolerant hybrids (7007, 7009, and 7028) that are the subject of this patent application averaged 7.00 for heat tolerance. As discussed above, the heat tolerance scale goes from one (1) to nine (9), with one (1) the most susceptible and nine (9) very highly resistant as described above. In general, ratings of five (5) or below are unmarketable in a heat stress growth condition and represent

significant economic loss to the broccoli growers if such a level of heat stress reaction occurs in their broccoli fields.

The broccoli lines from study #3 were also comparatively analyzed for bead size, yield, head shape, extension and maturity. The results are presented in Table 7.

The bead size rating is on a scale of 1-5. A bead size rating of 5.0 represents very, very large beads. A bead size rating of 1.0 represents very, very small beads such as cauliflower beads. A bead size rating of 4.0 represents large beads. An ideal bead size rating is 3.0 to 4.0 with a maximum desirable rating of 3.7 to 3.8. A combination of bead size rating of 3.8 to 4.0 combined with a high bead size uniformity rating is also acceptable.

The yield rating is on a scale of 1 to 10 where a rating of 10 represents a maximum estimated yield for a particular trial. A yield rating of 8.0 compared to a yield rating of 7.0 represents an approximate increase in yield of 50%. While high yields are generally desirable, at the highest yield ratings, hollow core may develop undesirably. A combination of high yield, high heat tolerance (and, therefore head-holding ability) good extension and good uniformity are most desirable. The heat tolerant lines and hybrids of the invention generally exhibited high yields. The high yielding capacity of these lines and hybrids is thought to be due, in part, to an ability to keep increasing head size while maintaining desirable commercial characteristics under heat stress.

The head shape, extension and maturity ratings were on a 0 to 10 scale. Head shape is an important selection criterion for broccoli. Head shape ratings of 7.0 to 8.0 are most desired. A head shape score of 3.0 represents a completely flat to nearly concave head. A head shape score of 4.0 to 5.0 represents a small head not yet approaching a semi-dome. A head shape score of 6.0 represents a semi-dome shaped head. A head shape score of 7.0 represents a

good, solid dome. A head shape score of 8.0 represents a deep dome. A head shape score of 9.0 represents a very pointed dome in the shape of a Christmas tree.

Head extension is also an important selection criterion. Head extension is a comparative measurement of the distance between the broccoli head itself and the leaves surrounding the broccoli. If the head is surrounded by leaves, the head is difficult to harvest. Ideally, the head will be extended up above the leaves to permit easy harvesting of the broccoli. An extension rating of 3.0 represents a head that is buried fairly deep within the leaf canopy. A head extension rating of 5.0 represents a plant having a head which extends only slightly above the leaf canopy. A head extension value of 7.0 represents significant extension of the broccoli head out of the canopy. Commercially available broccoli line Marathon has a head extension rating of 6.5-7.0. The beat tolerant broccoli lines of this invention have an extension rating of generally around 7.3. An extension rating of less than 5.0 is undesirable because the head is surrounded by too many leaves making the broccoli difficult to harvest.

Maturity is also an important selection criterion. The smaller the maturity rating number the earlier the harvest date. The larger the maturity rating number the later the harvest date. A late harvest date is indicative of a line which takes longer to reach maturity and, therefore, longer to produce a commercially acceptable head. Generally, a smaller maturity rating number is preferred because the broccoli grower is able to harvest his/her crop sooner. Later maturing lines (with higher maturity rating numbers) are acceptable so long as they continue to produce commercially acceptable heads with a proper head size, coloring, head shape, etc.

Prior art commercially available line Marathon generally has a maturity rating of 5.5/6.0. In contrast, the heat tolerant broccoli lines of this invention generally have a maturity rating of around 6.2.

TABLE 5

Temperature Data for Study #3

Date	Temperature (°F)		
	Max	Min	Average
07/03	79	47	62
07/04	84	48	63
07/05	75	52	60
07/06	75	52	62
07/07	75	52	61
07/08	71	48	59
07/09	63	54	58
07/10	70	54	59
07/11	73	53	59
07/12	74	52	59
07/13	85	53	65
07/14	87	54	69
07/15	82	50	63
07/16	72	32	62
07/17	76	56	64
07/18	83	58	68
07/19	89	52	69
07/20	83	53	67
07/21	88	53	71
07/22	100	55	78
07/23	99	59	77
07/24	88	56	69
07/25	95	54	72
07/26	81	58	70
07/27	76	55	63
07/28	78	55	62
07/29	75	56	62
07/30	72	56	61
07/31	72	57	62
08/01	82	57	65
08/02	83	56	65
08/03	88	54	68
08/04	83	56	66
08/05	77	56	64
08/06	74	58	64
08/07	79	59	66
08/08	90	56	71

TABLE 5

Temperature Data for Study #3

Date	Temperature (°F)		
	Max	Min	Average
08/09	98	59	74
08/10	109	60	81
08/11	100	61	78
08/12	91	58	70
08/13	83	56	66
08/14	84	52	64
08/15	81	50	62
08/16	86	50	66
08/17	92	53	71
08/18	98	58	75
08/19	97	60	75
08/20	92	57	71
08/21	89	58	68
08/22	74	54	61
08/23	74	53	61
08/24	72	51	61
08/25	69	53	60
08/26	71	52	60
08/27	77	54	63
08/28	81	50	63
08/29	86	51	67
08/30	87	50	67
08/31	83	51	64
09/01	69	54	59
09/02	81	51	61
09/03	91	48	64
09/04	95	52	71
09/05	94	46	72
09/06	95	57	71
09/07	91	55	69
09/08	93	54	71
09/09	98	54	69
09/10	91	58	71
09/11	83	61	69
09/12	89	64	73
09/13	95	68	77
09/14	93	64	75
09/15	77	56	64

TABLE 5

Temperature Data for Study #3

Date	Temperature (°F)		
	Max	Min	Average
09/16	68	56	60
09/17	83	32	70
09/18	85	52	67
09/19	87	55	69
09/20	88	57	68
09/21	76	55	62
09/22	73	53	61
09/23	72	52	60
09/24	71	51	59
09/25	65	54	59
09/26	65	49	57
09/27	71	46	46
09/28	73	52	60
09/29	64	48	57
09/30	70	56	59
10/01	63	56	58
10/02	69	47	59
10/03	69	44	55
10/04	76	43	58
10/05	83	44	63
10/06	87	47	65
10/07	78	45	60
10/08	74	51	61
10/09	72	43	57
10/10	75	45	57
10/11	78	40	56
10/12	73	46	58
10/13	75	45	59
10/14	69	32	59
10/15	71	40	54
10/16	74	43	57
10/17	77	39	57

TABLE 6

Comparative Analysis: Study #3

#	ID	Heat Tolerance Rating (0-9)**
1	Marathon	1
2	Pinnacle	3
4	H7009	8
5	Premium Crop	4-
6	H7007	7+
8	H7028	6
9	Patriot	1
10	H7010	
11	Laguna	5
12	H7021R	5-
13	Montecristo	4.1
14	Greenbelt	3-
15	393-2-19/7770	5-
16	393-2-19/7778	6-
17	393-2-19/7861	6
18	Sultan	2.5
19	Tierra	6-
20	393-2-19/7864	
21	393-2-19/7865	4+
22	Everest	2
23	Liberty	2+
24	Marathon	3
25	7881/M7007	7-
26	7770-2/393-2-47	6
27	7770/7935	6
28	7770/7935	6
29	7770/7887	7-
30	Landmark	2
31	H7009	7
32	8092/7825	3
33	8092/7795	4+
34	8092/7883	
35	8030/7935	
36	8030/7914	

TABLE 6

Comparative Analysis: Study #3

#	ID	Heat Tolerance Rating (0-9)**
37	H7007	7-
38	Pinnacle	2+
39	Greenbelt	2
40	393-2-19/1692	7-
41	393-2-19/1524	

** 0 = Most susceptible
5 = Intermediate
9 = Most tolerant

TABLE 7

Comparative Analysis: Study #3

#	ID	Bead Size	Yield	Head Shape	Extension	Maturity
1	7770	3.5	7.3	---	---	---
2	7778	3.7	6.1	7.0	7.5	3.7
3	7861	3.7	4.0	7.0	7.0	5.5
4	7864	3.5	6.0	6.7	---	8.0
5	7865	3.6	7.0	6.0	7.2	7.0
6	7881	3.7	7.3	6.7	5.0	7.0
7	7887	3.8	6.7	7.0	7.7	6.7
8	7935	3.7	---	6.7	7.4	5.0
9	8092	3.5	7.7	6.0	5.3	7.0
10	7883	3.8	---	7.3	7.5	6.7
11	7914	3.6	---	5.7	7.6	6.0
12	Pinnacle	3.7	7.0	6.3	7.0	4.0

5

Comparative Analysis Study #4

In a fourth study, various broccoli lines were analyzed and characterized for heat tolerance. Daily high and low temperature measurements for study #3 are presented in Table 8. The maximum daily temperatures during study #4 were generally cooler than the maximum daily temperatures of study #3. In the fourth study, the maximum daily temperature was never greater than 92°F. In contrast, during study #3, the maximum daily temperature was greater than 95°F on several days.

The heat tolerance ratings for several broccoli lines analyzed during study #4 are present in Table 9. The heat tolerant broccoli lines of the invention consistently exhibited heat tolerance ratings of around 7.0. In contrast, the heat tolerant ratings for the commercially available lines for study #4 averaged around 5.0. The heat tolerance ratings for the commercially available lines were generally higher in study #4 than study #3 because the temperatures were cooler during study #4 than in study #3. Since the commercially available lines were exposed to generally cooler temperatures in study #4, the heat tolerance ratings for these lines were higher during study #4.

In study #4, the heat tolerant broccoli lines were also compared to commercially available lines regarding yield, bead size, head shape, extension and uniformity. The rating scale is the same as that for comparative study #3, Table 7. Uniformity represents a comparative measurement of the similarity between the various plants within a line. High uniformity is desired by growers because it allows them to maximize their harvest efficiency. A minimum uniformity rating of 6.0 to 6.5 is generally viewed as commercially acceptable. A rating of 8.0 represents highly uniform broccoli lines. Uniformity values less than 6.0 are generally viewed as commercially unacceptable. The results are presented in Table 10.

In addition to being heat tolerant, the lines of this invention consistently gave higher head shape and extension ratings than the commercially available lines.

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
05/01	66	50
05/02	65	49
05/03	66	52
05/04	70	41
05/05	83	43
05/06	79	49
05/07	76	51
05/08	75	42
05/09	74	41
05/10	79	42
05/11	74	50
05/12	75	54
05/13	79	42
05/14	68	46
05/15	71	42
05/16	76	42
05/17	82	46
05/18	78	48
05/19	69	51
05/20	67	52
05/21	82	46
05/22	81	49
05/23	75	52
05/24	80	52
05/25	80	53
05/26	75	51
05/27	78	51
05/28	75	51
05/29	61	52
05/30	73	53
05/31	75	51
06/01	70	52
06/02	65	53
06/03	68	48
06/04	68	51
06/05	80	52
06/06	80	53

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
06/07	75	43
06/08	77	44
06/09	75	44
06/10	81	48
06/11	81	47
06/12	85	51
06/13	84	52
06/14	82	58
06/15	80	57
06/16	84	52
06/17	84	49
06/18	88	51
06/19	84	52
06/20	84	50
06/21	79	54
06/22	86	53
06/23	83	52
06/24	85	54
06/25	83	52
06/26	83	49
06/27	95	49
06/28	99	54
06/29	98	55
06/30	87	51
07/01	87	52
07/02	85	52
07/03	87	52
07/04	87	52
07/05	87	52
07/06	87	52
07/07	87	51
07/08	88	52
07/09	88	52
07/10	90	52
07/11	90	52
07/12	90	53
07/13	91	55
07/14	90	55

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
07/15	87	53
07/16	86	54
07/17	88	54
07/18	89	54
07/19	89	54
07/20	89	54
07/21	89	53
07/22	89	53
07/23	87	54
07/24	87	54
07/25	89	54
07/26	89	54
07/27	89	53
07/28	89	54
07/29	90	54
07/30	88	54
07/31	90	54
08/01	90	54
08/02	88	54
08/03	88	54
08/04	88	54
08/05	90	53
08/06	91	54
08/07	90	55
08/08	90	54
08/09	90	55
08/10	88	55
08/11	85	55
08/12	86	54
08/13	85	54
08/14	86	54
08/15	87	54
08/16	88	54
08/17	87	54
08/18	86	54
08/19	86	54
08/20	86	53
08/21	85	52

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
08/22	85	53
08/23	88	53
08/24	87	53
08/25	87	53
08/26	87	54
08/27	88	53
08/28	89	53
08/29	89	53
08/30	86	54
08/31	87	54
09/01	89	53
09/02	88	53
09/03	88	53
09/04	88	54
09/05	88	54
09/06	86	54
09/07	86	54
09/08	86	53
09/09	86	53
09/10	86	53
09/11	86	53
09/12	85	52
09/13	84	53
09/14	85	53
09/15	85	52
09/16	84	52
09/17	84	52
09/18	83	52
09/19	84	51
09/20	84	51
09/21	86	50
09/22	86	50
09/23	85	51
09/24	86	52
09/25	84	53
09/26	82	51
09/27	83	53
09/28	83	51

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
09/29	84	52
09/30	85	51
10/01	84	50
10/02	82	50
10/03	83	50
10/04	83	50
10/05	84	50
10/06	82	50
10/07	80	49
10/08	80	48
10/09	81	49
10/10	80	48
10/11	79	48
10/12	79	48
10/13	80	49
10/14	79	48
10/15	78	47
10/16	78	47
10/17	80	46
10/18	78	46
10/19	76	47
10/20	75	48
10/21	76	47
10/22	77	46
10/23	75	47
10/24	77	46
10/25	75	48
10/26	74	46
10/27	74	45
10/28	71	45
10/29	73	44
10/30	72	43
10/31	73	43

TABLE 9

Heat Tolerance Data for Study #4

#	ID	Heat Tolerance Rating*
1	4243-1	7.1
2	4263-1	7.2
3	4267-1	7.0
4		7.2
5	4274-1	6.9
6	4274-2	6.9
7	4278-1	7.4
8	4284-1	7.2
9	4285-1	6.8
10	4308-2	6.8
11	4309-1	6.7
12	4318-1	6.3
13	4320-1	7.0
14	4320-2	7.0
15	4321-1	7.1
16	4354-1	7.2
17	4354-2	7.2
18	4355-1	6.5
19	4377-1	7.1
20	4395-2	6.5
21	4412-1	6.9
22	4430-1	7.4
23	4432-1	6.9
24	4437-1	7.0
25	4450-1	6.6
26	4450-2	6.6
27	4460-1	7.1
28	4462-1	7.2
29	4465-1	7.3
30	4476-1	7.1
31	Pinnacle	5.0
32	Marathon	5.0
33	Greenbelt	4.5
34	7007	7.0
35	4201	7.0
36	4208	3.0
37	4209	6.0
38	4212	6.1
39	4219	7.0
40	4221	6.9

TABLE 9

Heat Tolerance Data for Study #4

#	ID	Heat Tolerance Rating*
41	4237	6.2
42	4280	6.1
43	4287	6.1
44	4288	7.3
45	4289	6.0
46	4290	6.7
47	4291/4459	7.2
48	4301	6.5
49	4303	7.1
50	4304	7.1
51	4317	6.4
52	4338	4.9
53	4370	6.2
54	4415	6.7
55	4418	5.5
56	4441	6.3
57	4442	6.3
58	4468	6.8
60	4470	6.5

* 0 = Most susceptible
5 = Intermediate
9 = Most tolerant

TABLE 10

Yield, Bead Size, Head Shape, Extension and Uniformity Data for Study #4

#	ID	Yield	Bead Size	Head Shape	Extension	Uniformity
1	4243-1	8.8	3.6	7.0	5.5	6.5
2	4263-1	7.0	3.4	7.3	7.3	6.8
3	4267-1	6.8	3.9	6.8	7.1	6.9
4	4267-1	6.9	3.9	7.1	7.2	
5	4274-1	7.2	3.5	7.3	7.5	6.8
6	4274-2	7.2	3.5	7.3	7.5	6.8
7	4278-1	7.6	3.7	7.4	7.2	6.8
8	4284-1	7.3	3.5	7.4	7.5	6.9
9	4285-1	7.4	3.7	7.3	7.3	6.0
10	4308-2	7.0	3.8	6.7	7.9	6.5
11	4309-1	6.9	3.7	6.5	8.2	6.4
12	4318-1		3.6	7.5	7.6	6.9
13	4320-1	7.0	3.6	7.5	7.5	7.0
14	4320-2	7.0	3.6	7.5	7.5	7.0
15	4321-1	7.0	3.7	7.6	7.2	6.9
16	4354-1	7.2	3.6	7.4	7.2	7.1
17	4354-2	7.2	3.6	7.4	7.2	7.1
18	4355-1	6.8	3.9	6.1	7.8	6.5
19	4377-1	7.0	3.7	7.4	7.0	6.8
20	4395-2	7.0	3.7	6.8	7.2	6.8
21	4412-1	7.0	3.8	6.5	7.8	6.1
22	4430-1	7.1	3.3	7.0	7.6	6.9
23	4432-1	7.4	3.5	7.2	7.5	7.0
24	4437-1	7.3	3.6	7.4	7.3	6.1

TABLE 10

Yield, Bead Size, Head Shape, Extension and Uniformity Data for Study #4

#	ID	Yield	Bead Size	Head Shape	Extension	Uniformity
25	4450-1	7.5	3.6	7.1	7.2	6.5
26	4450-2	7.5	3.6	7.1	7.2	6.5
27	4460-1	7.3	3.7	7.1	7.0	7.0
28	4462-1	7.2	3.5	7.3	7.3	6.7
29	4465-1	7.4	3.5	7.2	7.1	6.2
30	4476-1	7.8	3.5	7.4	7.1	6.5
31	Pinnacle	7.2	3.6	6.0	7.2	6.9
32	Marathon	7.3	3.3	6.0	7.0	6.7
33	Greenbelt	6.9	3.5	5.0	7.0	6.9
34	7007	7.5	3.8	7.3	7.1	7.0

TRANSGENIC BROCCOLI

The broccoli varieties of this invention can be transformed with useful genes to make heat tolerant transgenic broccoli varieties. Such useful genes include "terminator genes", herbicide resistant genes, insect resistant genes, virus resistant genes and the like.

To introduce isolated genes or a group of genes into the genome of plant cells such as broccoli an efficient host gene vector system is necessary. The foreign genes should be expressed in the transformed plant cells and consistently transmitted (somatically and sexually) to the next generation of cells produced. The vector should be capable of introducing, maintaining and expressing a gene in plant cells, from a variety of sources, including but not limited to plants and animals, bacteria, fungi, yeast or virus. Additionally it should be possible to introduce the vector into a wide variety of plants. The location of the new gene in the plant genome may be

important in determining effective gene expression of the genetically engineered plant. In addition, to be effective, the new gene must be passed on to progeny by normal breeding.

Directed genetic modification and expression of foreign genes in dicotyledonous (broad-leafed) plants such as tobacco, broccoli, potato and alfalfa has been shown to be possible using the T-DNA of the tumor-inducing (Ti) plasmid of Agrobacterium tumefaciens. Using recombinant DNA techniques and bacterial genetics, foreign pieces of DNA can be inserted into T-DNA in Agrobacterium. Following infection by the bacterium or Ti plasmid, the foreign DNA is inserted into the host plant chromosomes, thus producing a genetically engineered cell and eventually a genetically engineered plant. A second approach is to introduce root-inducing (Ri) plasmids as the gene vectors.

Transformation of broccoli is well known in the art of molecular biology. For example, in Cao, et al. "Transgenic broccoli with high levels of Bacillus thuringiensis CrylC protein control diamondback moth larvae resistant to CrylA or CrylC," Molecular Breeding 5:131-141 (1999), discloses one method of generation of transgenic broccoli. Such procedures can readily and easily be followed to produce transgenic heat tolerant broccoli plants.

ISOLATION OF HEAT TOLERANCE GENES

Now that heat tolerant broccoli plants have been identified and isolated, the identification of the gene or genes involved in heat tolerance is a straightforward process. One of ordinary skill in the art can identify genes involved in heat tolerance by comparing the DNA of heat tolerant and heat sensitive broccoli plants. One such method of isolating heat tolerance gene is the use of a matrix mill available from Cornell University in Ithaca, New York. The use of such a device greatly facilitates the isolation of heat tolerant genes. The device is capable of breaking up 96 small tissue samples simultaneously in sodium hydroxide, releasing the tissue's DNA and denaturing the protein. After the extraction, the tissue sample is then neutralized and the DNA is simultaneously diluted. Once diluted, the DNA is ready for analysis. Using the matrix mill one can compare several heat tolerant to several heat sensitive broccoli lines simultaneously.

In addition to using the matrix mill, basic molecular biological techniques may be utilized by one of ordinary skill in the art to isolate the heat tolerant broccoli gene. Such procedures are outlined in detail in Ausubel, et al. (Eds) (1987) "Current Protocols in Molecular Biology," John Wiley and Sons, New York.

Once the heat tolerant gene or genes are identified in broccoli, the corresponding heat tolerant gene or genes can be isolated in other plants through various hybridization techniques as described in Ausubel, et al.

Furthermore, biological material can be isolated from the seeds and plants of this invention by procedures well known in the art. Such material may include but is not limited to DNA, RNA, protein and carbohydrates.

DEPOSIT INFORMATION

Representative of, but not limiting the invention, Applicants have deposited seeds from M7028, M7007, M7009 and 393-2-19 with the American Type Culture Collection.

Applicants have made available to the public without restriction a deposit of at least 2500 seeds of broccoli M7028 with the American Type Culture Collection (ATCC), Rockville, MD 20852 which has been assigned ATCC number 203530.

Applicants have made available to the public without restriction a deposit of at least 2500 seeds of broccoli M7007 with the American Type Culture Collection (ATCC), Rockville, MD 20852 which has been assigned ATCC number 203531.

Applicants have made available to the public without restriction a deposit of at least 2500 seeds of broccoli M7009 with the American Type Culture Collection (ATCC), Rockville, MD 20852 which has been assigned ATCC number 203532.

Applicants have made available to the public without restriction a deposit of at least 2500 seeds of broccoli 393-2-19 with the American Type Culture Collection (ATCC), Rockville, MD 20852 which has been assigned ATCC number 203533.

The deposits will be maintained in the ATCC depository, which is a public depository, for a period of 30 years, or 5 years after the most recent request, or for the effective life of the patent, whichever is longer, and will be replaced if a deposit becomes nonviable during that period.

Although the foregoing invention has been described in some detail by way of illustration and examples for purposes of clarity and understanding, it will be obvious that certain modifications and alternative embodiments of the invention are contemplated which do not depart from the spirit and scope of the invention as defined by the foregoing teachings and appended claims.

We claim:

1. A heat tolerant broccoli plant.

2. Seed produced from the plant of claim 1.

3. Progeny seed produced from crossing the broccoli plant of claim 1 with another plant.

4. Broccoli plants produced from the seed of claim 3.

5. A broccoli seed capable of germinating into a plant which produces a commercially acceptable broccoli head under heat stress growth conditions.

6. The broccoli seed of claim 5 wherein said heat stress growth conditions are such that a broccoli variety selected from the group consisting of Marathon, Pinnacle, Premium Crop, Patriot, Laguna, Montecristo, Greenbelt, Sultan, Tierra, Laguna, Fiesta, Liberty and Landmark does not produce a commercially acceptable head.

7. The broccoli seed of claim 5 wherein said heat stress growth conditions include exposure of said plant to a maximum temperature of at least 90°F for at least 5 consecutive days during the growth cycle of said plant.

8. The broccoli seed of claim 5 wherein said heat stress growth conditions include exposure of said plant to a maximum temperature of at least 95°F for at least one day during the growth cycle of said plant.

9. The broccoli seed of claim 5 wherein said heat stress growth conditions include exposure of said plant to a maximum temperature of 85°F for at least 15 days during the growth cycle of said plant.

10. A broccoli plant or its parts produced by the seed of claim 5.

11. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 10.

12. A seed from said plant of claim 10 or its progeny.

13. A broccoli seed designated 393-2-19 and having ATCC Accession Number 203533.

14. A broccoli plant or its parts produced by the seed of claim 13.

15. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 14.

16. Progeny seed produced from crossing the plant of claim 14 with another plant.

17. Tissue culture according to claim 15 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

18. A broccoli plant regenerated from regenerable cells of a tissue culture according to claim 17.

19. A broccoli plant having all the phenotypic characteristics of a plant produced from the seed of claim 13.

20. A seed from said plant of claim 19 or its progeny.

21. A broccoli plant produced from the progeny seed of claim 16.

22. A broccoli seed produced from the broccoli plant of claim 20.

23. A broccoli seed designated M7028 and having ATCC Accession No. 203530.

24. A broccoli plant or its parts produced by the seed of claim 23.

25. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 24.

26. Tissue culture according to claim 24 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

27. A broccoli plant regenerated from regenerable cells of a tissue culture according to claim 26.

28. A broccoli plant having all the phenotypic characteristics of a plant produced from the seed of claim 23.

29. A seed from said plant of claim 24 or its progeny.

30. Progeny seed produced from crossing the plant of claim 24 with another broccoli plant.

31. A broccoli plant produced from the progeny seed of claim 30.

32. A broccoli seed produced from the broccoli plant of claim 31.

33. A broccoli seed designated M7007 and having ATCC Accession No. 203531.

34. A broccoli plant or its parts produced by the seed of claim 33.

35. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 34.

36. Tissue culture according to claim 35 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

37. A broccoli plant having all the phenotypic characteristics of a plant produced from the seed of claim 33.

38. A seed from said plant of claim 34 or its progeny.

39. Progeny seed produced from crossing the plant of claim 34 with another plant.

40. A broccoli plant produced from the progeny seed of claim 39.

41. A broccoli seed designated M7009 and having ATCC Accession No. 203532.

42. A broccoli plant or its parts produced by the seed of claim 41.

43. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 42.

44. Tissue culture according to claim 43 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

45. A broccoli plant having all the phenotypic characteristics of a plant produced from the seed of claim 41.

46. A seed from said plant of claim 42 or its progeny.

47. Progeny seed produced from crossing the plant of claim 42 with another plant.

48. A broccoli plant produced from the progeny seed of claim 47.

49. A broccoli seed selected from the group consisting of those broccoli seeds designated H7008, H7022, 393-2-47, 98-2192, 98-2088, 98-2061, H7007 and H70028.

50. A broccoli plant or its parts produced by the seed of claim 49.

51. A seed from said plant of claim 50 or its progeny.

52. A seed produced from crossing the plant of claim 50 with another broccoli plant.

53. A broccoli seed selected from the group consisting of those broccoli seeds designated: M7028, M7007, M7009, M7022, 393-2-19, H7008, H7022, 393-2-47, 98-2192, 98-2088, 98-2061, H7007, H7028, H7010, and H7021R, 4243, 4221, 4441, 4274-1, 4274-2, 4278-1, 4284-1, 4285-1, 4354-1, 4354-2, 4377-1, 4318-1, 4320-1, 4320-2, 4321-1, 4437-1, 4476-1, 4462-1, 4308-2, 4309-1, 4355-1, 4412-1, 4301, 4303, 4304, 4317, 4468, 4470, 4471, 4263-1, 4430-1, 4450-1, 4450-2, 4432-1, 4267-1, 7861, 7864, 7865, 7881, 7887, 7935, 8092, 7883, 7914, 7770, 7778, 4201, 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1, 4415, 4418, 4395-2.

ABSTRACT OF THE DISCLOSURE

Heat tolerant broccoli plants and seed produced therefrom are described. The heat tolerant broccoli plants are capable of producing a commercially acceptable broccoli head under heat stress growth conditions. The heat tolerant broccoli plants are exemplified by seeds deposited with the American Type Culture Collection and having ATCC Accession numbers: 203530, 203531, 203532, and 203533.

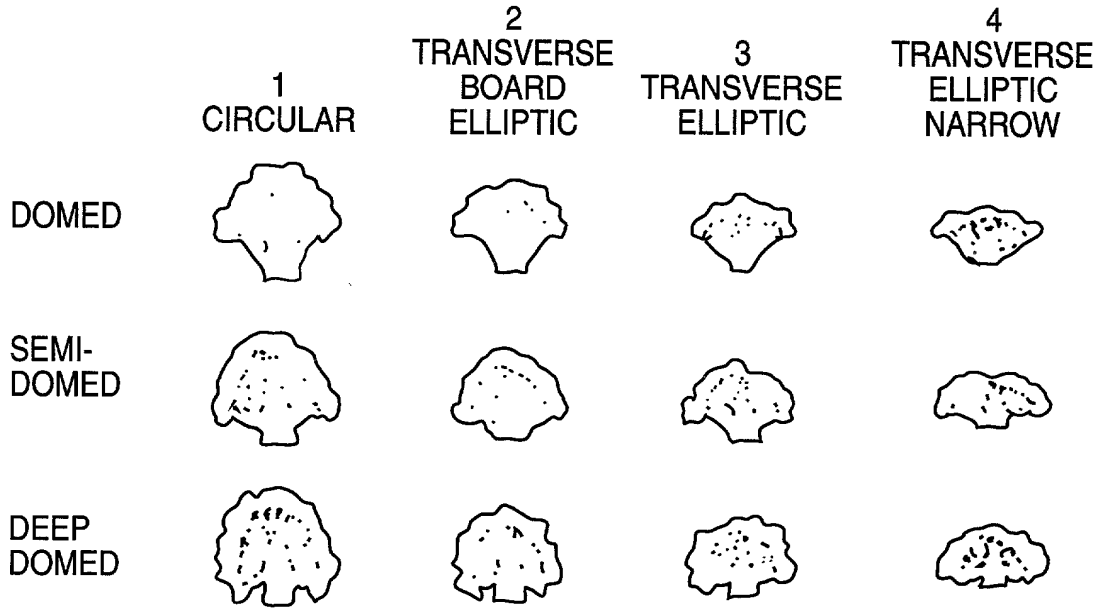

1
CIRCULAR
2
TRANSVERSE
BOARD
ELLIPTIC
3
TRANSVERSE
ELLIPTIC
4
TRANSVERSE
ELLIPTIC
NARROW
DOMED
SEMI-
DOMED
DEEP
DOMED

FIG. 1

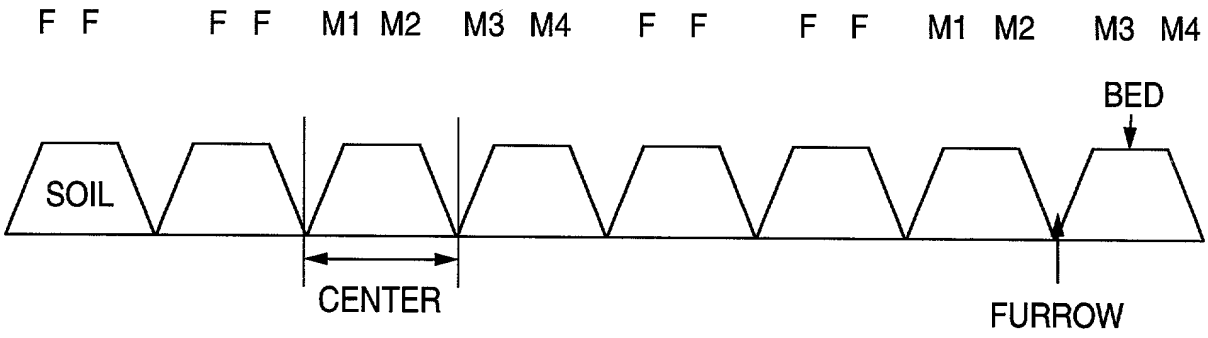

F F
F F
M1 M2
M3 M4
F F
F F
M1 M2
M3 M4
BED
SOIL
CENTER
FURROW

FIG. 2

PATENT
Docket No. 50085-20001.01

DECLARATION FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, WE HEREBY DECLARE THAT:

Our residence, post office address, and citizenship are as stated below next to our names.

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled: HEAT TOLERANT BROCCOLI, the specification of which is attached hereto unless the following box is checked:

☒ was filed on 29 December 1999 as International Application No. PCT/US99/31230 and was amended on _____ (if applicable).

WE HEREBY STATE THAT WE HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

We acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

We hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Application No.	Country	Date of Filing (day/month/year)	Priority Claimed?
			☐Yes ☐No

We hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
60/114,038	29 Dec. 1998

We hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, we acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status
09/328,121	08 June 1999	☐Patented ☒ Pending ☐Abandoned

We hereby appoint the following attorneys and agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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